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Haemoflagellates and Intestinal Flagellates From Anura of Louisiana.

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HAEMOFLAGELLATES AND INTESTINAL FLAGELLATES FROM ANURA OF LOUISIANA

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ABSTRACT

This study was undertaken to attempt to add to the knowledge of the almost unknown haemoflagellates and intestinal flagellates in Louisiana Anura.

A total of 503 frogs and toads, representing 21 species, was collected and examined from 1953 to 1958.

The hosts were collected from 30 different localities throughout the state. However, the largest number was collected in the vicinity of Baton Rouge and adjacent areas.

Sixteen species of flagellates have been identified and studied. One of these has been described as a new species.

The haemoflagellates were stained with Giemsa. The intestinal flagellates were stained and impregnated with iron-haematoxylin and silver-protein.

The sixteen species of flagellates are as follows: Trypanosoma karyozeukton, Trypanosoma rotatorium, Retortamonas dobelli, Chilomastix caulleryi, Karotomorpha swezyi,

Monocercomonoides elegans n. sp., Monocercomonoides melolonthae, Monocercomonas batrachorum, Tritrichomonas augusta, Tritrichomonas batrachorum, Trepomonas agilis, Urophagus intestinalis (?), Hexamitus intestinalis, Octomastix batrachorum, Octomitus neglecta, Trimitus parvus.

New information is presented on the biology of these flagellates in regard to their morphology, taxonomy, ecology and distribution.

A section is given to the discussion of host-parasite relationships. In addition, a check list of the haemoflagellates and intestinal flagellates is presented with the number of species of frogs and toads infected by each parasite.

INTRODUCTION

Although several studies of helminth parasites of amphibian hosts have been made in Louisiana, there has been no investigation of the protozoan parasites of the Anura of the state. The present study was undertaken in an effort to gain some knowledge of the parasitic protozoan fauna in the frogs and toads found in Louisiana.

Examination of 503 anurans belonging to twenty-one species and seven genera has given much information on the kinds of organisms parasitizing the Anura of Louisiana. Sixteen different species of Protozoa have been found in this study, one of which has not previously been described. Studies were conducted on living flagellates whenever possible. Iron-haematoxylin was utilized as a standard stain. However, the investigation was based on a great extent on the results obtained through the use of silver-protein impregnation inasmuch as many of the flagellates had never before been demonstrated by this technique. This technique has revealed morphological details of

considerable interest which heretofore have never been depicted. The study was limited to haemoflagellates and intestinal flagellates.

Field collections were begun in February, 1953, and were carried out intermittently until May, 1958. Collections were made, in one location or another, in every month of the year.

The main body of this report is given to a consideration of the species of parasites encountered. In listing hosts for any one protozoan, only those species actually found to be infected have been included. For all species of haemoflagellates and intestinal flagellates encountered, the description given is based on observations of the protozoan collected, whether they represent forms previously described or new species. The description is followed, when possible, by a discussion of what has been learned about the biology of the specific protozoan. One of the most important factors in this study was the study of the morphology of the intestinal flagellates with the aid of the silver-protein technique. In addition, the author related the findings with the available literature and determined the taxonomic relationship of the parasites. All drawings were made from specimens collected in this project.

Literature on haemoflagellates and intestinal flagellates in Louisiana is nonexistent. The literature on haemoflagellates and intestinal flagellates in general is scattered. The scattered condition of the literature on both haemoflagellates and intestinal flagellates has made the study extremely difficult and this factor has continued to be a handicap.

Reference and discussion of these papers has been deferred to the sections of the dissertation where it is considered applicable.

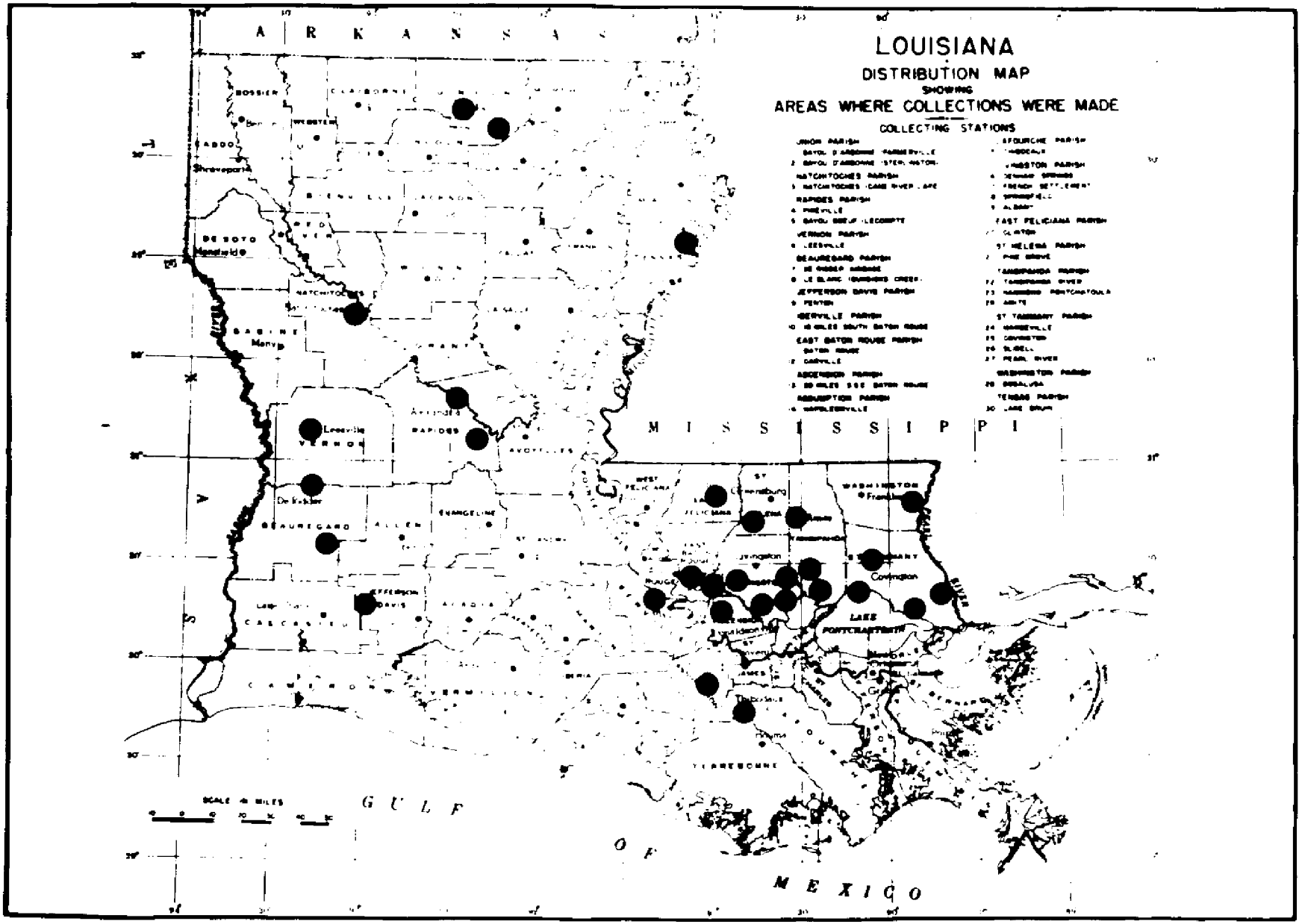
MATERIALS AND METHODS

The frogs and toads used in this study were obtained from eighteen Louisiana parishes: Union, Natchitoches, Rapides, Vernon, Beauregard, Jefferson Davis, Iberville, East Baton Rouge, Ascension, LaFourche, Livingston, East Feliciana, Saint Helena, Tangipahoa, Saint Tammany, Washington and Tensas. These specimens included Rana areolata Baird and Girard; Rana catesbeiana Shaw; Rana clamitans Latreille; Rana grylio Stejneger; Rana palustris LeConte; Rana pipiens sphenocephala Cope; Hyla avivoca Viosca; Hyla cinerea cinerea (Schneider); Hyla crucifer crucifer Wied; Hyla femoralis Latreille; Hyla gratiosa LeConte; Hyla squirella Latreille; Hyla versicolor versicolor LeConte; Pseudacris ornata (Holbrook); Pseudacris nigrita triseriata (Wied); Microhyla carolinensis (Holbrook); Acris gryllus crepitans Baird; Bufo fowleri Hinckley; Bufo terrestris (Bonnaterre); Bufo valliceps Wiegmann; Scaphiopus holbrookii holbrookii (Harlan). A total of 503 frogs and toads representing twenty-one species were examined.

Collection of Hosts: The specimens were obtained from night-collecting by means of an electric flash lamp attached to the author's head. The hands were left free to grasp the animals and at the same time a beam of blinding light could be directed at the animal. Specimens were placed in small wet cloth bags. In this way they could be transported with little mortality. If time did not permit an immediate examination for parasites, the animals were placed in small glass aquaria equipped with screened covers. The covers were movable to facilitate the handling of the specimens. All species were separated from each other, each kept in a separate aquarium. The specimens were checked within a 120-hour period after capture.

Area of Collection: Collections were made in thirty separate areas in the state of Louisiana (see map). The range of collection extended from Bayou D'Arbonne (Farmerville) to Thibodaux, and from Leesville to Pearl River. Reference to this map will show that not all of the state is equally represented. Most of the anuran hosts were collected in East Baton Rouge Parish and adjacent parishes. But a limited number of species of hosts were collected from localities throughout the state.

The various habitats from which the anuran hosts


LOUISIANA
DISTRIBUTION MAP
SHOWING
AREAS WHERE COLLECTIONS WERE MADE
COLLECTING STATIONS
ARKANSAS
MISSISSIPPI
GULF OF MEXICO
BOSSIER
CADDO
Shreveport
DE SOTO
Mansfield
SABINE
Many
NATCHITOCHES
Leesville
VERNON
DeRidder
BEAUREGARD
RAPIDES
Alexandria
AVOYELLES
EVANGELINE
ALLEN
JEFFERSON DAVIS
CALCASIEU
CAMERON
Baton Rouge
Covington
LAKE PONTCHARTRAIN
TERREBONNE
SCALE IN MILES

were collected differed greatly in an ecological way. They were taken from drainage ditches, damp grassy woodland, lakes, rivers, bayous, swamps, marshes, isolated ponds, and piney woodland. However, no effort was made to study particular habitats from which the hosts were collected. No restrictions were made on the localities of the anuran hosts studied. The purpose of this study was to collect as many of the kinds of frogs and toads as possible so that an overall picture of anuran protozoan parasites could be obtained for the state.

The fact that some species of Anura are much more widespread and are more easily collected than others accounts for the lack of balance in the number of species examined. A list of the species and the number of individuals per species examined for protozoan parasites in the present study is found in Table I.

Examination of Hosts: An animal was pithed and then examined for blood and intestinal flagellates. The blood from each specimen was examined for living haemoflagellates. Blood smears stained with Giemsa's stain were studied. Observations of the blood parasites were accomplished in the following manner: A sterile 2 cc. syringe using number 24 and 26 hypodermic needles was used to draw the blood from

TABLE I

CHECKLIST OF THE HOST SPECIMENS EXAMINED DURING THIS STUDY

Hosts	Number Examined
Rana areolata areolata Baird and Girard.	15
Rana catesbeiana Shaw.	38
Rana clamitans Latreille	36
Rana grylio Stejneger.	17
Rana palustris Le Conte.	4
Rana pipiens sphenocephala Cope	33
Hyla avivoca Viosca.	40
Hyla cinerea cinerea (Schneider)	32
Hyla crucifer crucifer Wied.	25
Hyla femoralis Latreille	3
Hyla gratiosa Le Conte	11
Hyla squirella Latreille	2
Hyla versicolor versicolor Le Conte.	10
Pseudacris ornata (Holbrook)	1
Pseudacris nigrita triseriata (Wied)	40
Microhyla carolinensis (Holbrook).	17
Acris gryllus crepitans Baird.	50
Bufo fowleri Hinckley.	20
Bufo terrestris (Bonnaterre)	20
Bufo valliceps Wiegmann.	38
Scaphiopus holbrookii holbrookii (Harlan).	50
	503

each host. The needle was carefully inserted into the pericardial region of the animal, then directly into the heart, and the blood was slowly drawn into the syringe. The needle was then quickly withdrawn and a drop of blood was immediately transferred to a sterile cover glass, which was then inverted to form a hanging drop. The cover

glass was placed in a depression slide and sealed with vaseline. In this manner it was possible to observe the organisms for a long period in the living state. The hearts of minute frogs were directly excised from the body and smears made directly onto cover glasses. Because of the small quantity of blood in the minute frogs it was impossible many times to observe the haemoflagellates in the living state, although they were subsequently observed in Giemsa stained blood smears.

For the examination of the living intestinal flagellates, the following procedure was employed. The intestine was carefully uncoiled and segments of the large and small intestines were excised and placed on a slide to which was added 0.65 per cent salt solution. By use of needles and forceps, the intestinal contents were evenly smeared and the cover glass added. The preparations were ringed with melted vaseline to prevent evaporation. Intestinal flagellates so prepared could be kept alive for 72 hours or longer.

For the permanent preparations, thin smears were made on cover glasses. Fixation was carried out at room temperature for twenty minutes to one-half hour in Hollande's fluid or modified Bouin's solution. Fixation

in Schaudinn's solution with or without acetic acid was carried out at 65 degrees C. for ten to twenty minutes.

Staining and Mounting: The stain used for the haemoflagellates was Giemsa stain. Intestinal flagellates were stained with Heidenhain's iron-haematoxylin and Moskowitz's (1950) modification of the Bodian silver-protein technique. One of the most significant factors in this study was the utilization of Moskowitz's modification of the silver-protein technique. Inasmuch as the present study is to a great extent based on the results obtained by the use of this technique, the author feels that attention should be called to some of the difficulties encountered with the method. The steps entailed in the utilization of the silver-protein technique are as follows: The first step necessitates the use of Mallory's bleach. Honigberg (1947) described the basic procedure entailed in the use of this bleach for protozoa. The application of Mallory's bleach is essential for impregnation of the parasites by the silver ions. According to Moskowitz, Mallory's bleach consisting of 0.5 per cent $KMnO_4$ and 5 per cent oxalic acid is successful. The author found that much of the smear was lost using the concentration specified by Moskowitz. The author proceeded

to reduce the percentages of both solutions so that this loss would be minimized. The least amount of material lost in this phase was found to occur in solutions consisting of 0.15 per cent $KMnO_4$ and 1.0 per cent oxalic acid. This was particularly true of the larger protozoan parasites such as in the trichomonad and monocercomonad groups. This loss is based on the comparisons of iron-haematoxylin smears from the same individual from which the parasites were taken. Honigberg (1951) states that the strength of $KMnO_4$ in itself affects the loss of the material. He states that he used 0.25 per cent $KMnO_4$. He found that smears taken from a strong solution of $KMnO_4$ and then immersed in oxalic acid of a given strength will cause the loss of much material. The author concurs with Honigberg on this matter. It is the opinion of the author that both $KMnO_4$ and oxalic acid denature the serum-like mucoid substance found naturally in the intestinal contents of a host. This mucoid substance acts as a cohesive, causing adhesion of the parasites to the cover glass. When this substance is denatured, the parasites are washed off more readily.

The second step entails the impregnation of the protozoa by the silver ion. It was found that the percentage

of silver ions in relation to the protein in solution affected impregnation. In addition, time of impregnation and temperature at which it was conducted affected impregnation of the parasites. The author found that 2.0-2.5 per cent silver-protein solution at 37 degrees C. for 12 hours gave best results.

Moskowitz specifies that best results are obtained with 1 per cent silver-protein solution for 36 hours or more at approximately 35 degrees C. Honigberg (1951) states that he used 0.5-1.0 per cent silver-protein solution, and that he obtained excellently impregnated organisms at this concentration when the temperature of impregnation was at 37 degrees C. and time of impregnation was four to six hours. On other occasions he found that 48 hours of exposure with 1 per cent solution had to be employed at a temperature of 37 degrees C.

The author found that the pH of the silver-protein solution profoundly affected the impregnation of the various protozoan parasites. Best results were obtained at a pH of 6.8-7.2 using N/10 NaOH as a buffer. The pH was measured by the use of the Beckman pH meter.

The third step entails the reduction of the silver ion. This was accomplished by using a hydroquinone

solution as indicated by Moskowitz (1950). Direct immersion of the smears from the silver-protein solution to the reducing solution produced more intense impregnation so that Moskowitz's distilled water washings between silver-protein and hydroquinone solutions were deleted from the procedure. Reduction was found to occur in 2-4 minutes.

The fourth step involves a solution of yellow gold chloride, Moskowitz (1950). This solution is utilized for the toning of the silver-impregnated protozoa. The solution was found to give the best results when the strength of the gold chloride was 0.1 per cent and the time of exposure to the solution was 1.5 to 3.00 minutes. A 0.1 per cent solution at 1.5 minutes and 25-30 degrees C. was found effective for Hexamitus, Octomitus, Urophagus, and Trimitus species; however, a 0.1 per cent solution at 3.00 minutes was found more effective with organisms of the trichomonad and monocercomonad groups.

The fifth step consists of immersion into a solution of oxalic acid. The longer the period of immersion in this solution, the less intense the impregnation. A 1.0 per cent solution for two minutes produced the most effective results with all protozoan parasites encountered.

The sixth step consists of immersion in a solution

of sodium thiosulfate. A 4.0 per cent solution at 4-5 minutes produced the most effective result. The longer the period of immersion in this solution, the less intense the impregnation. A final washing of 10 minutes in distilled water was found sufficient to remove excess sodium thiosulfate.

The smears were then dehydrated and mounted in balsam.

All drawings were made with the aid of the camera lucida.

TAXONOMY AND BIOLOGY OF THE HAEMOFLAGELLATES AND INTESTINAL FLAGELLATES OF ANURA FROM LOUISIANA

This section pertains to the individual description and discussion of each haemoflagellate and intestinal flagellate encountered during this study. Each parasite is either briefly or fully described. Some of the flagellates have been adequately described by others, and therefore it is only necessary to include brief descriptions of such forms. In other cases, previous descriptions were not adequate or the forms studied here have differed from those depicted so that more detailed descriptions were required. The most important single factor in this study is the description of individuals based on silver-protein-impregnated specimens. The use of this staining method has not been applied to many of the species encountered, and therefore the method has revealed morphological features which heretofore have not been described.

Succeeding the description of each species, a section is devoted to a discussion of the taxonomy, biology, and ecology of the particular protozoan parasite under consideration.

Trypanosoma karyozeukton Dutton and Todd, 1903

(Plate I, Figs. 1-8)

Host: *Acris gryllus crepitans*

Description: The host found to be parasitized by this haemoflagellate during this investigation included the above anuran only. Of the fifty of these hosts examined, only five were found to be infected. All infected hosts were collected at University Lake on the Louisiana State University campus during the months of March and April. Observations made on this haemoflagellate showed it to be monomorphic.

The body of the living haemoflagellate is elongated and slender. Living specimens show a relatively narrow undulating membrane. The nucleus is refractile and is large and conspicuous. It lies in the posterior half of the organism. Granules can be seen in the refractile nucleus. Myonemes running longitudinally in the periplast of the haemoflagellate may be seen in some specimens. The blepharoplast is very distinct in the living individual, appearing as a refringent granule a short distance from the posterior end. It appears to be round and is relatively large. Vacuoles are situated in all parts of the body.

They are very conspicuous in the living haemoflagellate. A free flagellum extends from the anterior end of the organism.

Movements of the living haemoflagellate are of three types: wriggling, circling, and progressive. When wriggling they twist over and over in S-like curves, appearing at the initial glance like a writhing knot. Seen on the slide in a drop of blood, the haemoflagellates progress rapidly in a definite direction, with the flagellum directed forward. The body twists and bends from side to side in its progressive movement. On some occasions the haemoflagellates were seen to move in a circling fashion. The haemoflagellates progress, stop, and then circle in one spot, after which they thrust themselves forward and proceed.

The undulating membrane is distinctly seen and shows extraordinary rippling movements. The progression of the haemoflagellates is effected chiefly by twists and turns of the body, aided by the movements of the undulating membrane. The flagellum appears to function as a guiding apparatus.

Giemsa-stained specimens show the organism to be elongated and slender (Fig. 3). The organisms range in length from 51.0 to 82.5 microns. The width ranges from

3.7 to 7.5 microns. The flagellum measures 9.25 to 26.0 microns in length.

The nucleus is large and conspicuous, lying in the posterior half of the organism. It frequently extends completely across the body of the organism and is almost always surrounded by an area stained more lightly than is the remainder of the body (Figs. 1, 7). Chromatic granules occur in the nucleus of some individuals (Figs. 5,8). Average measurement of the nucleus from 50 specimens show a width of 2.0 and a length of 6.0 microns.

Myonemes are seen in the periplast of some individuals (Fig. 6). The haemoflagellate possesses an axoneme which arises at the blepharoplast (Fig. 1), traverses the cytoplasm for a short distance, and then passes along the distal border of the undulating membrane, which in turn arises from the edge of the body as a thin ridge of cytosomal protoplasm (Fig. 2).

Giemsa-stained individuals show the blepharoplast to be large and spherical. It stains very intensively and more brilliantly than either nucleus or flagellum. In many individuals this structure is surrounded by a clear, halo-like area (Fig. 1). The distance between the blepharoplast and the nucleus varies relative to the length

of the specimen, averaging approximately 9.5 microns in 50 specimens.

In many instances, Giemsa-stained specimens show the cytoplasm as vacuolated (Figs. 5, 8). Other individuals show it as granular or coarsely reticular (Figs. 2, 4). Both ends of the organism are attenuated. Fixed and stained haemoflagellates are in many instances coiled with the posterior end innermost and the anterior end free (Figs. 6, 7, 8).

Comments: The description presented here agrees in all essential details to that given by Dutton and Todd (1903, 1907). The question arises as to whether or not this form is in actuality a form of Trypanosoma rotatorium. Wenyon (1926) states that the following trypanosomes described and recorded from Anura and as being distinct species are in actuality synonyms of Trypanosoma rotatorium. They are Trypanosoma mega Dutton and Todd, 1903; Trypanosoma karyozeukton Dutton and Todd, 1903; Trypanosoma rotatorium var. nana Ed. and Et. Sergent, 1905; Trypanosoma nelspruitense Laveran, 1905; Trypanosoma belli Nabarro, 1907; Trypanosoma Borelli Marchoux and Salimbeni, 1907; Trypanosoma hylae Franca, 1903; Trypanosoma leptodactyli Carini, 1907; Trypanosoma innominatum Pittaluga, 1905;

Trypanosoma somaliense Brumpt, 1906; Trypanosoma bocagei Franca, 1911; Trypanosoma bogagei var. parva and magna Mathis and Leger, 1911; Trypanosoma chattoni Mathis and Leger, 1911; Trypanosoma tumida Averinzev, 1918.

Laveran and Mesnil (1912) separate the trypanosomes of Anura in four categories. They separate from Trypanosoma rotatorium and Trypanosoma inopinatum the species Trypanosoma leptodactyli of Leptodactylus ocellatum of Brazil, and the trypanosomes found in toads.

Franca (1925) states that Trypanosoma mega and Trypanosoma karyozeukton of Bufo regularis are distinct species.

Kudo (1946) points out that identification is difficult and that it is better and safer to hold that all species of Trypanosoma reported from frogs belong to either Trypanosoma inopinatum or to Trypanosoma rotatorium until their life cycles are known.

Mayr (1957) states that "a species definition postulates at least a discontinuity and under the most favorable conditions, a triad of characteristics: Reproductive isolation, ecological difference, and morphological distinguishability."

The conditions and circumstances under which this

form was found supports Mayr's concept. Fifty Acris gryllus crepitans, seventeen Microhyla carolinensis, six Rana pipiens sphenocephala, three Rana clamitans, and four Bufo terrestris taken from the same area were found free of trypanosomes. Microhyla carolinensis, Rana pipiens sphenocephala, Rana clamitans, and Bufo terrestris did not harbor the morphological forms designated as Trypanosoma karyozeukton. Dutton and Todd (1903), (1908) reported this form from Bufo regularis and Rana nutti.

Morphologically, the shape and the posterior position of the nucleus and the location of the blepharoplast is very good evidence for designating this form as distinct from Trypanosoma rotatorium. The position of the nucleus of Trypanosoma rotatorium and the shape of the nucleus differs vastly from that of the form Trypanosoma karyozeukton.

Nigrelli (1945) states that culturing the haemoflagellate may throw light on specificity. He states that characteristics such as shape and size of the colonies, the time it takes the colonies to develop, cyclic forms that may occur, and nutritional requirements would indicate species differences.

He also states that transfaunation experiments and serological tests may give some evidence as to the validity of the described species of haemoflagellates.

Trypanosoma rotatorium (Mayer) 1843

(Plate II, Figs. 9-12)

Syn. *Amoeba rotatorium* Mayer, 1843
Trypanosoma sanguinis Gruby, 1843
Monas rotatoria Lieberkühn, 1870
Undulina ranarum Lankester, 1871

Host: *Rana areolata*, *Rana catesbeiana*, *Rana clamitans*, *Rana grylio*, *Rana pipiens*, *Rana palustris*, *Hyla cinerea*, *Hyla crucifer*, *Hyla avivoca*, *Pseudacris nigrita*

Description: This markedly polymorphic trypanosome was taken from the blood of the above-mentioned hosts collected in the vicinity of Farmerville, Sterlington, Cane River Lake, Leesville, DeRidder Airbase, Bundick's Creek, Fenton, Baton Rouge, Napoleonville, Denham Springs, French Settlement, Springfield, Albany, Clinton, Amite, Covington, Lake Bruin, Louisiana (see map).

Four morphological types of this trypanosome were seen in the blood of the hosts. One form is a large, compact trypanosome measuring 40 to 62 microns in length by 11 to 20 microns in breadth, with a highly developed undulating membrane. The nucleus, which is spherical, lies at the center of the body. The axoneme begins at the kinetoplast, passes along the border of the prominent undulating membrance, and terminates as a short flagellum of 4

to 20 microns in length. Myonemes, which run longitudinally in the periplast, may be seen in the form. The kinetoplast is posterior, lying half way between the posterior end and the centrally-placed nucleus (Fig. 9). Another form is a geometrically near-perfect spherical organism measuring 24 to 42 microns in diameter. The cytoplasm is highly granulated, and many vacuoles are present therein. At the outer margin of the body a narrow undulating membrane may or may not be seen encircling the organism. The kinetoplast, when present, is large and compact and may lie to one side. The nucleus, lying directly in the center, is spherical and stains lightly with Giemsa stain. There is no flagellum present (Fig. 10). A third type is an amoeboid-like form measuring 20 to 40 microns in length by 15 to 30 microns in breadth. The nucleus is inconspicuous but, when seen, is spherical with a peripheral ring of chromatin present. The kinetoplast, which is also many times inconspicuous, is spherical and large and may or may not be surrounded by a clear zone. The axoneme, when present, is well developed. The undulating membrane is poorly developed, or may be entirely absent in this stage. There is no flagellum. The periplast is weakly developed, which may account for the amoeboid-like movements and the

shape taken by this form. The cytoplasm is highly vacuolated and no internal myonemes are seen (Fig. 12). The last type is a large, compact form measuring 30 to 50 microns in length by 20 to 30 microns in breadth. The nucleus is ovoid and lies at the center of the organism. The axoneme begins at the kinetoplast, passes along the border of the prominent undulating membrane, and then terminates immediately at the anterior end of the body. There is no flagellum present. The myonemes are prominent and run longitudinally in the periplast. The kinetoplast is posterior and may be fractured. The cytoplasm is highly granulated and may or may not have vacuoles present (Fig. 11).

Trypanosoma rotatorium shows considerable movement but relatively little motility. The large, compact forms have a very active undulating membrane; however, there is relatively little progression of the organism itself. Contraction and expansion of the body occur, although this action is not obvious. In this particular form, where myonemes were evident, it was noted that these same myonemes become broader in character when the organism is at its thickest width and shortest length. These contractions of the myonemes appeared to originate from both ends

and travelled inward toward the nucleus. The organism then relaxed and began to lengthen itself. The geometrically near-perfect spherical organisms were noted to have little or no progression. All movements of this particular form were produced by either the very narrow undulating membrane which encircled the organism or by the body itself. The undulating membrane movement is extraordinarily slow and weak, producing little, if any, progression. Movements in the amoeboid form are through a combination of movements of lobe-like pseudopodia of Amoeba and the undulating movement of the body itself. The periplast is weakly developed.

Comments: That Trypanosoma rotatorium is polymorphic is well known. The extreme polymorphism demonstrated by this species has led to the description of new species by many investigators.

According to Wenyon (1926), Gluge (1842) probably was the first to have seen what was probably a haemoflagellate in the blood of anuran hosts. Wenyon states that Mayer (1843) saw and described forms of the same organisms under the name Amoeba rotatorium and that Gruby (1843) gave a better description, suggesting the new name Trypanosoma sanguinis. This trypanosome was reported by

other observers, and Lieberkühn (1870) proposed the name Monas rotatoria, and Lankester (1871) the name Undulina ranarum. Wenyon states that these were evidently the forms studied by Mayer. The trypanosomes of Anura have been studied by many observers in different parts of the world and, because of their polymorphism, numerous names appeared which are undoubtedly synonyms.

Nöller (1913) studied the entire question and came to the conclusion that only two species were represented among the large number of trypanosomes described from Anura. These two species were Trypanosoma rotatorium (Mayer, 1843) and Trypanosoma inopinatum Sergent, 1904. He described three forms of Trypanosoma rotatorium from the green frog, Rana esculenta. He obtained a good growth in sealed preparations of infected frog's blood mixed with an equal amount of bouillion. He was able to follow the development of the trypanosomes from Rana esculenta. According to him, the form found in the tadpole and also young frogs was characterized by a sharp-pointed posterior end and a long, slender, eel-like body. The second form was a large, broad form, often oval or round in shape with striated periplast. It was sluggish in its movements. The posterior end was often rounded, and the flagellum was

short or lacking. The third form was described as having a smooth, unstriated posterior end and a thin and flat body. The last two forms were found only in adult frogs, although the form found in the tadpoles was found in the adult. It was in this order that Nöller stated that these forms appeared in the blood of the host. He also states that infections in the adult frogs, Rana esculenta, may be superimposed by a second one with inoculation of infected blood of tadpoles of this host-species, or by injection of large doses of cultured T. rotatorium. Hyla arborea was also successfully infected by Nöller with T. rotatorium. This suggests that T. hylae Franca (1908) may be T. rotatorium. A species of toad, Bominator igneus, was used by him in transmission experiments. The species was inoculated with very large doses of T. rotatorium and no infection took place. He states that this species of toad has never been found naturally infected with a trypanosome. He performed similar experiments with the tortoise and the goldfish. Transmission experiments with these two species also gave negative results. This indicates a natural immunity and some degree of host specificity on the part of the parasite.

Fantham, Porter, and Richardson (1942) found a

trypanosome, which they designated as T. inopinatum, in the host Rana catesbeiana. They reported the presence of T. rotatorium in the same individual host. The form designated as T. inopinatum by them may be a young stage in the life cycle of T. rotatorium. In this country T. clamatae Stebbins, 1907, and T. parvum Kudo, 1922, are designated as young stages of T. rotatorium by Nigrelli (1945).

Nigrelli (1945) states that culturing the haemoflagellates may throw light on specificity and that transfaunation experiments and serological tests may give evidence as to the validity of the described species of haemoflagellates.

Retortamonas dobelli (Bishop) 1931

(Plate II, Figs. 13-14)

Syn: Embadomonas dobelli Bishop, 1931

Host: Rana catesbeiana, Rana clamitans, Rana pipiens, Pseudacris nigrita, Acris gryllus crepitans, Bufo fowleri, Bufo terrestris, Bufo valliceps

Description: This intestinal flagellate was observed in smears made from the rectal contents of the hosts listed above. The host-species were collected in the vicinity of Baton Rouge, Carville, Denham Springs, Amite, and Pine Grove, Louisiana.

The flagellate was sparse in each of the host-species examined. The description of the species is based on both iron-haematoxylin and silver-protein treated specimens.

Iron-haematoxylin stained and silver-protein impregnated specimens show the form of this species to be variable; however, the general impression received is that the majority of the organisms are broadly ovoid with the posterior end tapering to a blunt point. The size ranges from 6 to 12 microns in length by 4 to 7.5 microns in breadth. The organism is broadly rounded anteriorly, and its greatest diameter is in the area directly behind the

nucleus. It tapers abruptly at the posterior end (Fig. 13). Some individuals show a sharp caudal spike (Fig. 14).

Iron-haematoxylin and silver-protein specimens show two flagella which are unequal in length. They appear to arise from two minute basal granules, which in turn appear to originate at the peripheral region of the spherical nucleus facing the cytostomal pouch (Figs. 13, 14). The anteriorly-directed flagellum is as long as or longer than the body, whereas the cytostomal flagellum is shorter and thicker and protrudes from the cytostomal pouch.

The nucleus, situated at the most anterior part of the flagellate, is ovoid to spherical and varies from 1.5 microns to 2.5 microns in diameter. It has a distinct nuclear membrane, upon the inner surface of which can be seen minute chromatin granules arranged in such a manner that they appear as a single thin layer (Figs. 13, 14).

Along the ventral surface of this species is a large cytostomal pouch which extends approximately half the length of the body. The cytostomal lips are well differentiated and are supported by marginal fibers, as in Chilomastix, but the minute size of the organism makes it often extremely difficult to determine this point with accuracy. The cytostome appears as a twisted trough, which

is deepest at its anterior aspect. At times, it is overhung by a protoplasmic hood.

The cytoplasm is vacuolated in iron-haematoxylin stained specimens; however, silver-protein impregnated individuals show it as granular.

Comments: Mackinnon (1911, 1912, and 1915) described two species of intestinal flagellates from trichopteran and tipulid larvae. These two species became the basis for Mackinnon's new genus, Embadomonas. Numerous other species have been described and placed in this genus, including the above species.

Wenrich (1932) stated that Retortamonas gryllotalpae described by Grassi (1879) from Gryllotalpa gryllotalpa, is cogeneric with Mackinnon's Embadomonas, and therefore, has priority over Embadomonas. This connection is accepted. The species of flagellate described here is placed in the genus Retortamonas (Grassi) Wenrich as Retortamonas dobelli. Evidence for placing the genus Retortamonas Grassi 1879 is stated by Wenrich, 1932.

This is the first report of the species from Anura of Louisiana. Bishop (1931) reported this species from Rana temporaria, Bufo vulgaris, and Salamandra maculosa; and Wenrich recorded it from Rana catesbeiana, Rana

clamitans, Rana pipiens, Acris gryllus, and Bufo valliceps. Both authors fail to state the locality from which the host-species were collected.

Pseudacris nigrita, Bufo fowleri, and Bufo terrestris constitute new records as host-species for this intestinal parasite. This form is identified as Retortamonas dobelli from the combined descriptions of Bishop (1931) and Wenrich (1932).

Chilomastix caulleryi (Alexeieff) 1909

(Plate III, Figs. 15-20)

Syn: Macrostoma caulleryi Alexeieff, 1909

Host: Rana catesbeiana, Rana clamitans, Rana pipiens, Hyla cinerea, Hyla crucifer, Hyla avivoca, Pseudacris nigrita, Acris gryllus crepitans, Bufo terrestris

Description: This species was found in the rectal contents of the hosts listed above. The hosts were collected in the vicinity of Baton Rouge, Carville, Denham Springs, Springfield, Albany, Clinton, Amite, Covington, and Slidell, Louisiana.

The description of this parasite is based on 50 specimens taken at random from five different populations.

The living trophozoite is elongated and pear-shaped, measuring 10 to 46 microns in length by 6 to 20 microns in breadth. Individuals slowed down by methyl cellulose distinctly show the cytostomal flagellum. It is attached to the right cytostomal lip by an extremely thin undulating membrane which can be seen to vibrate in a typically undulating manner. This undulating membrane apparently acts as an organelle to move food particles into the posterior end of the cytostomal pouch. The organism has a very thin but relatively inflexible pellicle so that the

shape of the organism is generally maintained. Changes in shape and the appearance of amoeboid forms as reported by Chalmers and Pekkola (1918), Kofoid and Swezy (1920), and Boeck (1921) for Chilomastix mesnili were never observed in C. caulleryi.

The organism moves slowly and does not appear to progress in a jerky fashion as described for C. mesnili by many observers. The cytostomal pouch can be distinctly seen. It is the most striking feature of the organism. This structure begins a short distance below the anterior extremity and extends posteriorly for a distance of approximately one-fourth to three-fourths the length of the body, taking on a spiral course as it traverses posteriorly.

The cytoplasm is vacuolated and contains irregularly-shaped material which appears to be bacterial in nature.

Iron-haematoxylin and silver-protein preparations show the organism as pear-shaped with the posterior extremity usually assuming a spike-like point. They measure from 10 to 46 microns in length by 6 to 20 microns in breadth. The greatest diameter is generally just posterior to the level of the anteriorly placed nucleus.

At the anterior extremity of the body are seen

three to five basal granules. They appear as darkly-staining minute structures. Haematoxylin and silver-protein treated specimens show these granules clearly. They give rise to the three unequal anterior flagella, to the fibrils that form the borders of the cytostomal pouch, and to the cytostomal flagellum (Figs. 15, 16, 17). Usually two of the anterior flagella originate from the right anterior basal granule, whereas the other originates from the left anterior basal granule (Figs. 15, 17). At times each anterior flagellum appears to arise from an individual basal granule (Fig. 19).

The cytostomal pouch is characteristic of the genus. Iron-haematoxylin and silver-protein preparations show it as a large, elongated, and in many instances twisted, structure. It extends from one-fourth to three-fourths the length of the body, but may in some instances traverse the entire length of the body. It appears broadest at the most posterior portion. It is characterized by having two lips, which stain intensely with both iron-haematoxylin and silver-protein. Each lip is supported by a fibril which extends the entire length of the border of the lip. Silver-protein preparations show each cytostomal fibril taking its origin from an individual basal granule located at the side

of the spherical nucleus (Figs. 15, 17). Within the cytostomal pouch can be seen a long flagellum. This flagellum may be observed clearly in specimens impregnated with silver-protein. It arises from an individual basal granule situated between the two basal granules giving rise to the cytostomal fibrils (Figs. 15, 17).

The nucleus is spherical and is situated at the most anterior extremity of the organism. As many times as not the chromatin material within the nucleus is arranged in a plaque or plaques adhering closely to the nuclear membrane (Figs. 15, 16, 17, 18). In other instances the nucleus is filled with diffuse chromatin material assuming the shape of minute granular structures (Fig. 20).

Iron-haematoxylin preparations show the cytoplasm highly vacuolated and containing inclusions which appear to be bacterial in nature (Fig. 20). Silver-protein specimens show the cytoplasm as granular.

Some of the stained preparations as well as all of the living organisms show a "spiral groove" which Kofold and Swezy (1920) described for Chilomastix mesnili (Fig. 19).

Comments: The history of the nomenclature through

which the genus Chilomastix has passed has been adequately dealt with in detail by Chalmers and Pekkola (1918), Dobell and O'Connor (1921), and Wenyon (1926). Chilomastix caulleryi was originally described by Alexeieff (1909) as Macrostoma caulleryi from material collected from the intestines of tadpoles and in Axolotl. He found out later that the name Macrostoma was not available, so he transferred the flagellate to the genus Tetramitus Perty, 1852. It was later discovered, however, that these parasitic forms were not of the same type as the free-living Tetramitus; therefore, in 1912 Alexeieff erected the genus Chilomastix, in which genus this form is now placed. Since that time Chilomastix caulleryi has been redescribed by Dobell and O'Connor (1921) and Grassé (1926).

The other species of Chilomastix which has been described from Amphibia is Chilomastix giganta by Nie (1948), who found the species in the rectal contents of a salamander (Pseudotriton sp.).

The forms in this collection, stained by iron-haematoxylin, compare favorably to those described by Alexeieff (1909) as Macrostoma (= Chilomastix) caulleryi, by Dobell and O'Connor (1921) and Grassé (1926) as Chilomastix caulleryi.

An interesting observation arising from this study is that the Chilomastix populations may constitute size races. There appear to be two races--a small race and a large, robust race--both of which have only slight morphological variations within the race, so that size appears to be the main distinguishing racial feature. Because of the absence of distinct morphological differences, the two races are considered to be one species, Chilomastix caulleryi. Both races appear to be present within different populations of the same host-species and within intrapopulations of a particular host-species. The small race varies in size between 10 to 16.5 microns in length by 6 to 8.5 microns in breadth, whereas the large robust organisms vary in size between 20 to 46 microns in length by 10 to 20 microns in breadth.

That the large robust race is not Chilomastix giganta Nie is obvious. C. giganta has distinguishing features which set it apart from the large robust race of C. caulleryi. These characteristics are tabulated in Table II.

Although this species has been recorded from various amphibian hosts, this is the first report to record the parasite from Anura of Louisiana. In addition, specimens

TABLE II

COMPARISON OF THE FEATURES EXHIBITED BY THE SPECIES CHILOMASTIX CAULLERYI AND CHILOMASTIX GIGANTA

Distinguishing characteristics	C. caulleryi small race	C. caulleryi robust race	C. giganta
Size	width 6-8.5u (7.25u) length 10-16.5u (14u)	width 10-20u (12.5u) length 20-46u (32u)	width 7.1-14u (10.3u) length 28.6-57.3u (40.3u)
Flagella	3-subequal	3-subequal	3-equal
Anterior end of organism	bluntly rounded	bluntly rounded	terminates as a short snout-like projection
Nucleus	spherical to ovoid diffuse or plaqued	spherical to ovoid diffuse or plaqued	4-6u along long axis, chestnut shaped with large cap-like endosomal body at anterior pole
Cytostome	large, broad, 1/4-3/4 length of body	large, broad, 1/4-3/4 length of body	narrow, elongated pouch, 1/3-1/2 length of body

TABLE II (continued)

Distinguishing characteristics	*C. caulleryi* small race	*C. caulleryi* robust race	*C. giganta*
Basal granules	3-5, silver-protein impregnated	3-5, silver-protein impregnated	2-4, silver-protein impregnated
Host	frogs and toads	frogs and toads	salamander

stained in iron-haematoxylin and impregnated with silver-protein are described.

Karotomorpha swezyi (Grassé) 1926

(Plate IV, Figs. 21-26)

Syn: Polymastix bufonis Swezy, 1916
Tetramastix swezyi Grassé, 1926

Host: Rana catesbeiana, Rana clamitans, Rana pipiens, Bufo terrestris, Bufo fowleri

Description: These ovoid to pyriform flagellates were taken from the rectum of the above-mentioned hosts, collected in the vicinity of Baton Rouge, Carville, Denham Springs, Springfield, Clinton, Pine Grove, and Covington, Louisiana.

The living flagellate moves by short jerks without changing the shape of its body. The rigidity is due to the fact that the organism possesses a periplast which is thick and resistant. Four anteriorly located flagella whip about so rapidly and are so slender that they hardly are seen in the living specimens. When motility is slowed down by methyl cellulose, they can be counted very easily. A refractile nucleus is located at the anterior end of the organism. The cytoplasm is colorless and in most instances vacuolated. There is no cytostome present nor are food inclusions found in the cytoplasm. The size of the flagellate ranges from 5 to 16 microns in length by 4 to 9 microns

in breadth. The anterior end is broadly rounded or may, as in some specimens, be somewhat pointed. The posterior end is broadly rounded, blunt, or pointed. The thick periplast is marked by striations which extend obliquely across the body. These striations appear as ridges or folds in or on the periplast. They escape observation in living specimens and in preparations stained with iron-haematoxylin; however, they may be demonstrated without difficulty in silver-protein impregnated individuals. All individuals appear to have the striations lacking at the anterior extremity (Figs. 21, 23, 25, 27). Iron-haematoxylin-stained specimens and silver-protein-impregnated individuals reveal ovoid to spherical basal granules which lie near the anterior extremity of the body. There are two of these structures present in all non-dividing individuals (Figs. 21, 22). Swezy (1916) illustrated only one granule in the material studied by her; however, Tanabe (1925) depicted two particles. These granules give rise to the mastigont structures. Each basal granule gives rise to one long and one short flagellum (Figs. 21, 22, 25).

In iron-haematoxylin-stained specimens the nucleus varies from spherical to ellipsoidal and lies just posterior to the basal granules. It is comparatively large in

structure measuring from 1.5 microns to 3 microns in diameter. It has a distinct nuclear membrane with chromatin material diffused throughout the nucleus. Most specimens show a distinct endosome appearing as a large eccentric structure within the nucleus (Figs. 22, 26). Iron-haematoxylin-stained specimens show the endosome as a deep-staining structure eccentrically placed in the nucleus, at times adhering to the nuclear membrane. Silver-protein-impregnated individuals show the endosome as a dark structure eccentrically placed in a wide clear zone. This zone represents the non-impregnating nucleus when the silver-protein method is used.

Iron-haematoxylin-stained specimens reveal a dark-staining structure posterior to the nucleus and lying adjacent to it. The structure is exhibited as a ribbon or sausage-like structure when it is viewed from the side (Figs. 22, 24, 26). Silver-protein-impregnated individuals do not reveal this structure; therefore, this structure is not comparable to the parabasal bodies of trichomonad and tritrichomonad species.

In iron-haematoxylin specimens the cytoplasm is alveolar, and large vacuoles may or may not be present. Silver-protein-impregnated specimens show the cytoplasm

as granular. No cytostome could be demonstrated in either iron-haematoxylin-stained or silver-protein-impregnated specimens.

Comments: Confusion exists in the literature concerning the details of structure and identity of this flagellate. The most extensive account is that of Travis (1934) to which the author refers the reader for the full account of the history of taxonomy of this species.

Grassé (1926) stated that Tetramastix (= Karotomorpha) swezyi, observed by Swezy (1916) in Diemyctylus torosus, Batrachoseps alternatus, and Rana pipiens of U. S. A., is distinct from Tetramastix (= Karotomorpha) bufonis of Europe because "T. swezyi à sa cuticule striée obliquement et à son systeme blépharoplastique presque toujours réduit à un seul grain."

Swezy (1916) stated that she did not initially find striations in material which she identified as T. bufonis and because of this considered her species to be identical with T. bufonis. Subsequently, she found striations in the periplast of the specimens which she had initially examined, and therefore stated that Dobell (1909) and Alexeieff (1911) missed this characteristic in their

respective descriptions of the species. However, Grassé (1926) restudied the species bufonis in Europe and again was unable to detect striations; therefore, he named the American species Tetramastix (=Karotomorpha) swezyi. Travis (1934) states that if striations can be demonstrated on bufonis, then swezyi may prove to be a synonym. Inasmuch as the new silver-protein-impregnation method clearly brings out the striations without difficulty in the species Karotomorpha swezyi, this characteristic should be cleared up regarding the matter of whether Karotomorpha bufonis has striations in its periplast.

In this study the author observed a large, elongated, sausage-shaped structure partially surrounding the nucleus and many times extending posteriorly for some distance in the cytoplasm of the organism. Dobell (1909) neglected to describe this structure in bufonis; however, Alexeieff (1911), Swezy (1916), Tanabe (1925), and Grassé (1926) have described and illustrated it. Alexeieff (1911) named the structure "corps siderophile." Janicki (1915) homologized it with the parabasal apparatus in Trichonympha. Swezy (1916) specified it as the "parabasal body" and stated that it was connected to one of the basal granules by means of a fine fibril. In this study the author noted

that at some instances a fine fibril could be detected connecting the above structure to one or the other of the two basal granules; however, this was not the case usually and could only be detected in specimens stained with iron-haematoxylin (Fig. 26).

Parabasal bodies vary greatly within different protozoan organisms. The morphology, location, and physiology vary significantly. Usually the apparatus is connected with the basal granule complex and has a position close to the nucleus, although it is not directly affiliated with it. Kofoid and Swezy (1915) state that the parabasal body has its origin from the nuclear chromatin material and that it varies in size according to the metabolism of the organism. They state that it acts as a "kinetic reservoir." Duboscq and Grassé (1933) state that the structure is the Golgi apparatus because acetic acid destroys the parabasal apparatus and the Golgi complex, that both are demonstrable with the same technique, that the parabasal apparatus is made up of chromophile and chromophobe constituents as is the Golgi complex, and that the parabasal apparatus shows strong evidence that it is secretory in nature. Kirby (1931) showed that the parabasal apparatus could be stained with Delafield's haematoxylin and Mallory's triple stain

after fixation with fixatives containing acetic acid. He stated that the apparatus does not show any evidence to indicate that it may be an apparatus which is secretory in nature. Kudo (1946) states that not all so-called parabasal bodies are homologous or analogous.

The author found that this structure is brought out clearly in specimens fixed with Bouin's solution, with and without 5 per cent acetic acid. Acetic acid, therefore, does not destroy this structure. It is therefore not comparable to the Golgi apparatus. In addition, the structure is not brought out in silver-protein-impregnated specimens which show the parabasal bodies clearly in both Tritrichomonas augusta and Tritrichomonas batrachorum taken from the same individual host-species in which Karotomorpha swezyi was taken. It is therefore not comparable to the parabasal body of the tritrichomonad group.

A more comprehensive study of the physiology of the structure for this species is needed.

This is the first report of the species from Anura of Louisiana. Rana clamitans, Bufo terrestris, and Bufo fowleri constitute new records as host-species for this intestinal flagellate.

Monocercomonoides elegans n. sp.

(Plate V, Figs. 28-32)

Hosts: *Acris gryllus crepitans*, *Pseudacris nigrita*

Description: Among the anuran hosts examined in the present study, a species of *Monocercomonoides* was found in three specimens of *Pseudacris* and two specimens of *Acris*. As far as the author is aware, this is the first report of the genus from these host-species. All hosts were collected during February and March on the campus of Louisiana State University. The rectal contents of all hosts contained an abundance of this species.

The living trophozoite is pear-shaped to ovoid. Size in any given population does not vary significantly. The pear-shaped (pyriform) form is most frequently encountered. The cytosome of this species appears to be relatively inflexible, although the pellicle is thin. The organism moves rapidly with a progressive movement, rotating clockwise on its longitudinal axis. In the living specimen the three anterior flagella propel the organism forward. This is accomplished by the backward lashing of the flagella. A fourth, the trailing flagellum, is attached for the greater part to the cytosome of the

organism. It is passive, taking no part in the movement of the organism. An exceedingly thin refractile axostyle can be observed. It forms the longitudinal axis of the organism. The cytoplasm appears hyaline in nature.

Iron-haematoxylin and silver-protein-treated specimens show the organism as pyriform to ovoid in shape. They measure from the extreme anterior end to the tip of the axostyle 10 to 13 microns in length by 4 to 6 in breadth. Measurement of 100 individuals from five populations gave a range of 10 to 13 microns in length, with an average of 11.5 microns, by 4 to 6 microns in breadth, with an average of 4.5 microns. Measurements of the species are closely comparable in the two host species in which the organism was found.

The anterior end of the organism is rounded, whereas the posterior end terminates to a relatively sharp point. Iron-haematoxylin and silver-protein specimens show two large blepharoplasts at the anterior end of the body. The blepharoplasts are linked to one another by a connecting fibril. Iron-haematoxylin specimens show this connecting fibril as an exceedingly thin fibril, whereas silver-protein preparations depict the blepharoplastic fibril as extremely thick.

Nie's (1950) explanation of the location of the nucleus in relation to the axostyle sets up an arbitrary establishment of dorsal and ventral sides. He considers the axostyle as a medial structure forming the longitudinal axis of the organism. In this regard the nucleus is located ventral to the axostyle. With this in mind, he considers the two blepharoplasts in dorsal and ventral position.

Silver-protein preparations show three of the four flagella of equal length. They may or may not be directed forward. They are approximately the length of the body of the organism. The fourth or trailing flagellum is two to three times the length of the organism. The diameter of the trailing flagellum appears thicker than the three equal flagella. Each flagellum appears to end in a fine terminal filament (Fig. 30).

Usually the dorsal blepharoplast gives rise to the trailing flagellum, but on occasion it was noted to arise from the ventral blepharoplast. Two anterior flagella arise from the ventral blepharoplast, whereas the third arises from the dorsal blepharoplast (Figs. 28, 30).

The pelta is difficult to demonstrate in iron-haematoxylin-stained specimens; however, silver-protein-

stained individuals show this structure clearly. It is located at the anterior end of the organism and appears to originate as an extension of the cytosome. The most anterior border stains deeply with silver-protein.

The funis arises from either the dorsal or ventral blepharoplast. It appears as a thin, chromatic fibril which passes posteriorly along either the dorsal or ventral surface of the organism. It is approximately one-half to three-fourths the length of the body. At times this structure appears to be torn away from the body (Fig. 32). This structure is not discernible in iron-haematoxylin-stained specimens, but specimens stained with silver-protein depict it clearly.

The axostyle appears to originate from the dorsal blepharoplast. It is not differentiated into a capitulum and trunk; rather, it is a thin chromatic rod of equal diameter along its entire length. It forms the longitudinal axis of the organism. The posterior end of the axostyle projects for a short distance from the posterior end of the organism. This area impregnates intensely with silver-protein so that it appears as a spike-like structure similar to that of the tip of a fish hook (Figs. 29, 30, 32). Iron-haematoxylin specimens lack this manifestation.

The nucleus is spherical and is always located at the anterior end of the organism. The size of the nucleus varies from 2 to 2.75 microns in diameter with an average of 2.4 microns, according to measurements taken from 25 specimens. Within the nucleus is seen a large endosome which is depicted clearly in both iron-haematoxylin and silver-protein specimens. It is centrally located and relatively large. It may assume a shape of spherical to ovoid.

The cytoplasm appears vacuolated in specimens stained with iron-haematoxylin, whereas silver-protein specimens show it as granular.

No parabasal body is seen. Inasmuch as all specimens studied were impregnated heavily with silver, it is reasonably valid to conclude that no parabasal body exists in this species. Reproductive forms are rarely observed (Fig. 31).

Comments: Literature describing Monocercomonoides from anuran hosts is meager. Previous reports have described Monocercomonoides from insects, amphibia, reptiles, and mammals. Bishop (1932) reported a member of this genus from Bufo vulgaris. This is the only species known to occur in Anura and she designated it as Monocercomonoides (= Retortamonas) rotunda. The first forms to be described

were Monocercomonoides (= Retortamonas) gryllotalpae (Grassi, 1879), and Monocercomonoides (= Retortamonas) melolonthae (Grassi, 1879) in the intestinal contents of insects. Wenrich (1946) states that the genus Monocercomonoides Travis, 1932, has species in insects, amphibians, and mammals; however, he does not specify species of this genus within the amphibian host species, nor does he indicate the species of hosts in which the parasites are found. Few papers report members of this genus from reptiles. Descriptions of species from the genus Monocercomonoides are given by Tanabe (1933). Grassé (1926) described a species Monocercomonoides (= Monocercomonas) colubrorum from the intestinal contents of Tarentola mauritanica. Moskowitz (1951) states that he found Monocercomonoides in the intestinal contents Crotalus viridis viridis, Holbrookia propinqua propinqua, Iguana iguana rhinolopha, Phrynosoma cornutum cornutum, P. douglassii brevirostri, and Sceloporus sp. Kirby and Honigberg (1949) describe a species of Monocercomonoides which they designate as Monocercomonoides pilleata from the genus Citellus; and Gabel (1954) describes two species of Monocercomonoides, M. digranula and M. robustus, from Marmota monax.

The intestinal flagellates of the genus Monocercomonoides (= Retortamonas) are closely related to Monocercomonas (= Eutrichomastix). The genus possesses four flagella, one of which is a trailing flagellum. In place of the relatively thick, hyaline axostyle of Monocercomonas, Monocercomonoides possesses a fibre (axostyle) which stains deeply with iron-haematoxylin and silver-protein.

Differential characteristics which set the two genera apart are listed in Table III.

Of the described species, Monocercomonoides elegans does not resemble any species described from insects. M. elegans most nearly resembles M. lacertae (Tanabe, 1933), but differs insofar as it possesses a spike-like structure at the posterior portion of the axostyle when stained with silver-protein. M. elegans differs so vastly from M. (= Retortamonas) rotunda (Bishop, 1932) that morphological comparison is needless.

TABLE III

COMPARISON OF THE FEATURES EXHIBITED BY THE GENERA MONOCERCOMONAS AND MONOCERCOMONOIDES

Features	Monocercomonas	Monocercomonoides
Hosts	Insects, fish, amphibia, reptiles, mammals, birds	Insects, amphibia, reptiles, mammals
Size and shape	Ovoid to pyriform 6-17u length x 4-10u breadth	Ovoid to pyriform 4-13u length x 3-6u breadth
Flagella	(4) 3 anterior, equal, usually directed forward; 1 trailing, nonadhering to cytosome	(4) 3 anterior, equal, usually directed forward; 1 trailing, adhering to cytosome
Cytostome	Present in some species; probably functional	Not present
Axostyle	Flexible, hyaline rod, relatively thick, differentiated into capitulum and trunk	Thin chromatic rod of equal diameter along entire axis; posterior end may differentiate into spike-like structure
Pelta	Present	Present
Parabasal body	Present; disc or rod-shaped	Not present
Blepharoplast	4, from which all flagella arise	2, 1 giving rise to 1 anterior flagellum and trailing flagellum; 1 giving rise to 2 anterior flagella. Blepharoplasts united by a transverse filament
Funis	Not present	Present

Monocercomonoides melolonthae (Grassi) 1879

(Plate VI, Figs. 33-37)

Syn: Schedoaceromonas melolonthae Grassi, 1879
Monocercomonas insectorum Grassi, 1881

Hosts: Hyla versicolor, Hyla crucifer

Description: The species was collected from the rectal concents of the two hosts listed above. Individuals of both host-species were collected at Denham Springs, Louisiana, during the month of March. Observations on the species are based on fifty specimens; twenty-five stained with iron-haematoxylin and twenty-five by silver-protein impregnation. Specimens stained with iron-haematoxylin conform closely with the descriptions of Grassi (1879) and Travis and Becker (1931). A detailed description of the species based on iron-haematoxylin stained specimens is omitted. Description of individuals based on silver-protein impregnated specimens is warranted inasmuch as the use of this staining method has not been applied in the studies of Monocercomonoides from amphibia or insects. This method has revealed morphological features which heretofore have not been depicted.

Iron-haematoxylin and silver-protein stained individuals show the organism as ovoid although some specimens

may be pyriform (Fig. 37). The body appears rounded anteriorly and posteriorly with the caudal end of the axostyle flush with the posterior end of the cytosome.

Measurements of fifty individuals from four populations gave a range in length of 4.00 to 7.00 microns with an average of 5.75 microns. Breadth of the individuals ranged from 4.00 to 6.00 microns with an average of 5.50 microns. Iron-haematoxylin specimens show four flagella. As often as not however, it is difficult to count the number of flagella following this technique. Silver-protein impregnated specimens regularly show four flagella clearly. Three flagella are equal in length and are approximately the length of the body. The fourth or trailing flagellum may be two to two and one-half times the length of the body. It is closely applied to the cytosomal surface becoming free at approximately the posterior one-third of the body. Silver-protein stained specimens show the recurrent flagellum of a diamater equal to that of the anterior flagella. None of the flagella was observed to end in fine terminal filament. Iron-haematoxylin and silver-protein specimens show two blepharoplasts at the anterior end of the body. These blepharoplasts may or may not be closely adherent to the peripheral region of the

nucleus. The particles are widely separated from one another. Each blepharoplast gives rise to two flagella. One gives rise to two of the anterior directed flagella, the other gives rise to the third anterior flagellum and the trailing flagellum. The axostyle may originate from either of the two (Figs. 35, 36). Silver-protein specimens depict the two blepharoplasts joined by a transverse filament. This filament is not readily seen in iron-haematoxylin stained individuals. In a few specimens stained with silver-protein a filament is seen to arise from the blepharoplast giving rise to the recurrent flagellum. This filament traverses from the blepharoplast to the peripheral region of the nucleus (Fig. 36).

A small pelta may be seen in some specimens stained with silver-protein. It is located at the anterior end of the cytosome. This structure is not demonstrable in specimens stained with iron-haematoxylin (Figs. 35, 37). On occasion a funis may be seen in silver-protein specimens, however, the structure is so minute and slender that it is not easily seen. It appears to arise from the blepharoplast giving rise to the recurrent flagellum (Fig. 35).

The axostyle may originate from either blepharoplast.

It appears as a thin chromatic rod and forms the longitudinal axis of the organism. The posterior end does not project from the caudal end of the cytosome, but terminates flush with the posterior end of the organism.

The spherical nucleus is located at the very extreme anterior end of the cytosome. In iron-haematoxylin and silver-protein stained specimens is seen a large endosome. It is center in position. No parabasal body is present.

The cytoplasm appears vacuolated in iron-haematoxylin stained specimens, on the other hand silver-protein impregnated individuals show the cytoplasm as granular. Many specimens have large chromatic spheres within the cytoplasm. The origin and function of these spheres are unknown.

Comments: Although this species has been recorded from various insect hosts, this is the first report to record the parasite from anuran hosts.

In this study Monocercomonoides melolonthae (Grassi, 1879) is redescribed. Specimens impregnated with silver-protein and stained with iron-haematoxylin are described. This is the first report of M. melolonthae from Anura of Louisiana.

Monocercomonas batrachorum (Dobell) 1909

(Plate VII, Figs. 38-42)

Syn: Trichomastix batrachorum Dobell, 1909
Eutrichomastix batrachorum Dobell, 1909

Host: Rana clamitans, Rana grylio, Rana pipiens sphenocephala, Hyla crucifer crucifer, Hyla avivoca, Pseudacris nigrita triseriata, Acris gryllus crepitans, Bufo fowleri

Description: This organism has been identified in the present study from the hosts listed above. The mentioned host-species were collected in the vicinity of Baton Rouge, Carville, Denham Springs, French Settlement, Springfield, Clinton, Amite, Covington, Slidell, and Bogalusa, Louisiana. The protozoan parasite was found only in the rectal contents of the hosts. The description of Monocercomonas batrachorum is based on 100 specimens taken at random from ten different populations. Preparations were fixed with Schaudinn's fluid and Hollande's fluid and stained with iron-haematoxylin and silver-protein.

In life, the trophozoite appears as a typically hyaline body pyriform or ovoid in shape. They are actively motile, swimming in the fecal material with a progressive motility. The living organism may be readily measured when slowed down by the addition of methyl cellulose solution.

They measure from the extreme anterior end to the tip of the axostyle 6 to 16.6 microns in length by 4 to 9 microns in breadth. The number of flagella cannot be distinguished in the actively motile organism, but with the addition of methyl cellulose they are made out as delicate threadlike structures having their origin from the anterior end of the organism. No basal granules can be observed in the living individual. The flagella number four, three of which are directed anteriorly and move in a whip-like fashion producing the progressive motility of the organism. The fourth trails posteriorly and does not appear to aid in the motility, but trails passively along with the organism.

The living organism exhibits slightly to the right of the anterior end a pelta which appears as a crescent-shaped protoplasmic projection. This pelta is extensible and movement of the structure is similar to that of a pseudopodium.

A flexible hyaline rod-like axostyle extends along the medial axis of the organism beginning just posterior to the bases of the flagella, widening as it passes by or over the refractile nucleus, and tapering as it proceeds posteriorly to the end in a spike-like point beyond the

body of the organism.

The cytoplasm is vacuolated and as often as not contains irregular-shaped material the origin of which is unknown. The author has never observed the actual ingestion of food by this organism.

Stained specimens are 5 to 16 microns in length by 3 to 10 microns in breadth. At the anterior end of the body are seen two basal granules from which the four unequal flagella arise. The three anterior flagella which are directed anteriorly arise from a basal granule which is relatively larger and more anteriorly placed than the basal granule giving rise to the long posteriorly-directed flagellum (Figs. 38, 39). At times, the basal granules are depicted as a fused, irregular mass with the parabasal body having its origin posterior to it and connected by a thick fibril. Silver-protein-impregnated individuals show the four flagella arising from the base of the parabasal body or just anterior to the structure (Figs, 40, 41, 42).

In iron-haematoxylin-stained specimens, the axostyle is a relatively thick hyaline structure with thin chromatic borders. This structure commences just posterior to the basal granules, curving around the ovoid nucleus, and then traverses posteriorly through the axis of the organism,

terminating as a free,spike-like structure. At the emergence of the free portion of the axostyle can be seen narrow periaxostylar rings. Lateral to the periaxostylar rings are located deep-staining granules.

The pelta is situated at the extreme anterior end of the body, lying usually to the right of the axis of the body. Iron-haematoxylin specimens show the anterior border of the pelta as a thin black fibril. The proximal portion is obscure so that it is impossible to define it from the cytosome (Fig. 38). Silver-protein impregnated individuals depict the pelta as a cone-like extension which impregnates lightly except for the anterior-most border, which impregnates heavily with silver. The proximal portion of the pelta can at times be defined from the cytosome in this stain (Figs. 40, 41, 42).

The nucleus is ovoid. There is an endosome centrally placed. Chromatic material lies at the periphery of the nucleus (Fig. 38). Silver-protein specimens show the nucleus as a clear zone, the borders of which are lightly impregnated with silver. The endosome can be observed at the center of the clear zone, impregnated heavily with silver ions (Figs. 41, 42).

The parabasal body cannot be depicted by the iron-

haematoxylin method; however, silver-protein-impregnated specimens show the parabasal body as a rod-like structure having its origin at the basal granule complex and connected to this mass by a thick fibril. It extends along the lateral border of the nucleus for a distance of 2 to 3 microns, and often is observed to have a thin fibril extending from its distal portion. This is the parabasal fibril (Fig. 42).

The cytoplasm appears vacuolated in preparations stained with iron-haematoxylin; however, it assumes a granular appearance in silver-protein preparations.

Comments: The forms described in this study that had been stained in iron-haematoxylin agree in essential morphological details to those forms described by Dobell (1909) as Eutrichomastix (= Monocercomonas) batrachorum. The only apparent difference is that forms from this locality average slightly smaller than those described by Dobell. There are not sufficient differences for regarding the form found in this study as a distinctly different species. Two other species which are similar to that of Monocercomonas batrachorum are Monocercomonas colubrorum (Hammerschmidt, 1844) from Natrix sipedon pictiventris, and Monocercomonas mabuiae (Dobell, 1910) from Crotalus

viridis. Although superficially similar, they differ specifically in that Monocercomonas colubrorum possesses a parabasal body which appears ring-like, and Monocercomonas mabuiae possesses a parabasal body which appears disc-shaped. Both of these species, in addition to these morphological differences, are found in reptiles, as reported by Moskowitz (1951).

It is interesting to note that this flagellate is always associated with Tritrichomonas batrachorum. The flagellates of the genus Monocercomonas differ from those of Tritrichomonas in that they possess no undulating membrane, and the number of their flagella is constant, i.e., always four, in all species described.

Doflein (1916) stated that the two forms belonged to the same genus, that Monocercomonas is a phase in the life cycle of Tritrichomonas and not a distinct genus. Chatton (1920) stated that cultures of trichomonads taken from the guinea pig assume both trichomonad and monocercomonad types. He also states that when the monocercomonad types are injected into the body cavity of guinea pigs that the trichomonad forms reappear.

Reichenow (1918, 1920) observed that in lizards the circulatory system was contaminated by monocercomonads

from the intestinal contents. In one case he noted that a lizard had died of monocermonad infection, that at the time of death the only forms noted in the circulatory system were the monocercomonad forms; however, on the next day, in addition to monocercomonad forms, there were trichomonad forms. He therefore concluded that the trichomonads had derived from the monocercomonad form and that the two types are stages of one organism. Dobell (1907) regards Monocercomonas as a separate genus and states that degenerating monocercomonads have a tendency to assume trichomonad-like forms because the posterior trailing flagellum adheres to the body. Bishop (1931) cultured several strains of Tritrichomonas batrachorum, during which time she states that the organism did not undergo morphological variation and that no indication of an undulating membrane appeared. De Gupta (1936) followed Bishop's line of experimentation and went one step further. He isolated pure-line strains of Trichomonas from the grass snake and from the Boa, and one pure-line strain of Monocercomonas from another grass snake. He states that the cultures were under observation for three to ten weeks. He found that Monocercomonas did not develop an undulating membrane and may simulate a trichomonad if the bordering flagellum was accidentally

detached; and that Monocercomonas from the snake never developed an undulating membrane. From these observations he concluded that Trichomonas and Monocercomonas are generically distinct.

Although this species has been recorded from various amphibian hosts, this is the first report to record the parasite from Rana clamitans, Rana grylio, Rana pipiens sphenocephala, Hyla crucifer crucifer, Hyla avivoca, Pseudacris nigrita triseriata, Acris gryllus crepitans, and Bufo fowleri. In addition, specimens impregnated with silver-protein are described. Monocercomonas batrachorum possesses certain morphological characteristics revealed for the first time by the silver-protein technique. These morphological characteristics have been discussed.

Tritrichomonas augusta (Alexeieff) 1911

(Plate VIII, Figs. 43-49)

Syn: Trichomonas augusta Alexeieff, 1911

Host: Rana areolata, Rana catesbeiana, Rana clamitans, Rana grylio, Rana palustris Rana pipiens, Hyla cinerea, Hyla crucifer, Hyla avivoca, Hyla gratiosa, Hyla versicolor, Pseudacris nigrita, Microhyla carolinensis, Acris gryllus crepitans, Scaphiopus holbrookii, Bufo terrestris, Bufo fowleri, Bufo valliceps

Description: Tritrichomonas augusta is one of the more common intestinal flagellates of the hosts listed above. The flagellate was found in smears made from the rectal contents of hosts collected in the vicinity of Farmerville, Sterlington, Cane River Lake, Pineville, Le Compte, Leesville, De Ridder Airbase, Bundick's Creek, Fenton, Baton Rouge, Carville, Napoleonville, Thibodaux, Denham Springs, French Settlement, Springfield, Albany, Clinton, Pine Grove, Tangipahoa River, Hammond-Pontchatoula, Amite, Mandeville, Covington, Slidell, Pearl River, Bogalusa, and Lake Bruin, Louisiana.

In life, the flagellate appears as a typically hyaline body pyriform in shape. They are actively motile, swimming in the fecal material with a progressive motility. The number of flagella cannot be distinguished in the

actively motile organism; however, with the addition of methyl cellulose, they are made out as delicate thread-like structures having their origin from the anterior end of the organism. A refractile basal granule can be observed at the anterior end of the living organism. It is from this basal granule that the three anterior flagella originate. A broad undulating membrane is distinctly seen and shows extraordinary rippling movements. It appears to arise by means of a marginal filament from the refractile basal granule, and extends posteriad. It may or may not curve around the body. The amount of curvature varies with the individual. The undulating membrane terminates in the vicinity of the posterior end of the organism, whereas the marginal filament continues free as a trailing flagellum.

A thick hyaline axostyle traverses the longitudinal axis of the animal. It appears to originate at the basal granule and terminates as a conical tip at the posterior end of the organism. Along the margin of the hyaline axostyle are seen round refractile granules. These granules may or may not extend the entire length of the axostyle.

A refractile ovoid nucleus is seen to occupy a position in the middle one-third of the organism. It is usually to the left of the axostyle.

The cytoplasm is vacuolated and as often as not contains irregularly-shaped material.

Stained specimens are pyriform, measuring 15 to 32 microns in length by 5 to 17 microns in breadth.

There are three anterior flagella. Iron-haematoxylin specimens show all three of constant diameter throughout their length. Silver-protein preparations show knob-like enlargements at the distal ends of the flagella (Figs. 43, 44). The flagella appear to originate from the basal granule complex. The basal granules are located slightly posterior to the anterior extremity. Silver-protein-impregnated individuals show the area occupied by the basal granules as a spherical clear zone. The three anterior flagella, the costa, marginal, and accessory filaments of the undulating membrane appear to rise from this clear zone (Figs. 44, 45).

Iron-haematoxylin specimens show the axostyle as a thick hyaline structure with chromatic borders. Within the axostyle are seen dark-staining chromatic granules. These endoaxostylar granules are best seen in iron-haematoxylin specimens (Fig. 47). In silver-protein specimens they impregnate faintly (Figs. 43, 44, 45). On occasion they are absent and this may be the result of the metabolic

condition of the individual, for they do not appear in all individuals within the same population.

Iron-haematoxylin and silver-protein individuals show the undulating membrane thrown into folds as it extends along the body of the individual. At the base of the undulating membrane is the costa. It arises from the basal granule complex, extends posteriad, and terminates at or near the chromatic ring (Figs. 43, 44, 45).

The parabasal body is best seen in silver-protein preparations. It measures 5 to 7 microns in length by 0.3 to 1 micron in breadth. In some individuals the parabasal is rod-like in shape (Fig. 43), whereas in other specimens it is ribbon-like (Fig. 45). In still other individuals it takes on a vacuolated appearance (Fig. 44). Extending from the anterior region of the parabasal is a long parabasal fibril. In some specimens impregnated with silver-protein this fibril is missing, or, perhaps, the staining technique did not reveal it (Fig. 43). Other specimens show it clearly (Figs. 44, 45).

The nucleus is demonstrated in both iron-haematoxylin and silver-protein preparations, although less commonly in silver-protein. Iron-haematoxylin shows the nucleus bounded by a nuclear membrane. Both iron-haematoxylin and silver-

protein specimens show a large endosome situated near the center of the nucleus (Figs. 43, 47).

The cytoplasm is vacuolated in iron-haematoxylin specimens (Fig. 47); however, it appears granulated in individuals impregnated with silver-protein (Figs. 43, 44, 45).

Comments: The forms in the author's collection compare favorably with the descriptions given by Honigberg (1953) and Buttrey (1954).

Kofoid and Swezy (1915) stated that the kinetoplast is composed of two parts, the centrosome and the basal granule. Various investigators have stated that the kinetoplast consists of granules, each granule being associated with one of the mastigont structures. In specimens where special care was taken to destain critically, the flagella could be seen to arise from separate granules composing the kinetoplast complex.

The author also found a constant number of three flagella on individual specimens, which is in agreement with Buttrey (1954).

The rod-like appearance of the parabasal body has been described by Janicki (1915), Alexeieff (1917), Samuels (1941), and Honigberg (1950). Janicki, Alexeieff, and

Samuels used a fixative lacking acetic acid and haematoxylin as a stain. Honigberg utilized the silver-protein method. Duboscq and Grassé (1933) and Chen (1949), utilizing haematoxylin, reported a ribbon-like body. In addition, they reported that the parabasal consisted of two substances, chromophile and chromophobe, which accounts, in their opinions, for the uneven staining reaction of the parabasal body.

The author found these variations in specimens impregnated with silver-protein and is of the opinion that this variation is produced by the uneven deposition of the silver ion at the surface of the parabasal body.

Further reference and discussion of this species has been deferred to the section under comments of Tritrichomonas batrachorum, where it is considered applicable.

Tritrichomonas batrachorum (Perty) 1852

(Plate VIII, Figs. 46, 48, 49)

Syn: Trichomonas batrachorum Perty, 1852

Host: Rana catesbeiana, Rana clamitans, Rana grylio, Rana pipiens, Hyla cinerea, Hyla crucifer, Hyla avivoca, Pseudacris nigrita, Microhyla carolinensis, Acris gryllus crepitans, Scaphiopus holbrookii, Bufo fowleri, Bufo terrestris, Bufo valliceps

Description: Tritrichomonas batrachorum is a very common flagellate of the large intestine of the frogs and toads examined. The species was found in smears made from the intestinal contents of the hosts listed above, and collected in the vicinity of Pineville, Leesville, De Ridder Airbase, Baton Rouge, Carville, Napoleonville, Thibodaux, Denham Springs, Springfield, Albany, Clinton, Pine Grove, Tangipahoa River, Hammond-Pontchatoula, Amite, Mandeville, Covington, Slidell, and Bogalusa, Louisiana.

The living shape of the body is usually ovoid; however, it changes considerably from time to time, and under certain conditions pseudopodia are formed. The amoeboid activity is utilized for the attachment of the body to debris which the organism is associated with. This amoeboid activity may also be used in conjunction

with feeding activity.

The anterior end of the body is bluntly rounded, while the posterior end is more or less tapered.

The flagella may be observed in specimens slowed down by methyl cellulose. They are three in number and are as long as or longer than the body of the organism, exclusive of the protruding axostyle. They are seen to move from one side of the body to the other in sweeping movements very much like the action of a whip which is lashed to and fro. At times the proximal portions of the flagella appear adherent to one another or twisted so that they form a common stem. No basal granule can be seen in the living organism.

The axostyle appears to commence at the most anterior end of the organism, traverses through the longitudinal axis of the animal, and then protrudes from the cytoplasm as a sharply pointed caudal process. The caudal process moves from side to side. This movement appears to be the result of the contraction of the cytoplasm around the axostyle.

A narrow, undulating membrane is seen. It arises from the most anterior end, extends posteriad, and terminates approximately at the middle two-thirds of the body.

A trailing flagellum continues free from the posterior end of the undulating membrane.

A refractile ovoid nucleus is seen to occupy a position in the middle one-third of the organism. The cytoplasm is vacuolated and contains irregularly-shaped material.

Iron-haematoxylin and silver-protein preparations showed the organism to be ovoid, although some pyriform-shaped individuals were noted. The size varies from 7 to 14.5 microns in length by 4.5 to 11.5 microns in breadth.

Iron-haematoxylin specimens show all three anterior flagella of constant diameter throughout their length (Fig. 49). Silver-protein preparations show knob-like enlargements at the distal ends of the flagella (Figs. 46, 48). The lengths of the anterior flagella are unequal. The proximal portions of the flagella arise from a single basal granule situated at the anterior end of the organism (Fig. 49).

Iron-haematoxylin and silver-protein specimens show the axostyle as a thick hyaline structure. There are no endoaxostylar granules present. Both iron-haematoxylin and silver-protein preparations show the axostyle originating in the area of the basal granule and lying at the

longitudinal axis of the organism. It projects posteriad to form a long and tapering pointed rod (Figs. 48, 49). In some specimens a bulbous cytoplasmic sheath lies just anterior to the terminal end of the axostyle (Fig. 46). A crescent-shaped pelta lies near the anterior surface of the body (Fig. 48).

The nucleus is situated approximately in the middle one-third of the body. It is ellipsoidal or ovoidal and usually lies to one side of the axostyle (Figs. 46, 49). Usually it is filled with diffuse chromatin material; however, at times an endosome can be clearly seen situated at the center of the nucleus (Figs. 46, 49).

Silver-protein-impregnated specimens show the parabasal body as V-shaped and attenuated. It appears to arise from the basal granule (Figs. 46, 48). At times a parabasal filament of variable length can be seen to extend out from the distal portions of each arm of the V-shaped parabasal body (Fig. 48).

Iron-haematoxylin specimens show the cytoplasm as vacuolated, whereas silver-protein specimens assume a granular appearance.

Comments: Wenyon (1926) states that Perty (1852) specified that only one species of Trichomonas infected

frogs. Perty suggested the name Trichomonas batrachorum for this common flagellate, and his idea of only one species of trichomonad infecting the gut of frogs was formally accepted. Conversely, Alexeieff (1911) stated that two species of trichomonads could be recognized in Anura. He specified that these were T. batrachorum Perty and Trichomonas augusta.

Alexeieff and Gwéléssiany (1929) stated that Tritrichomonas augusta was restricted to toads and that Tritrichomonas batrachorum was found only in frogs. Lavier (1942) stated that Tritrichomonas augusta was equally seen in both frogs and toads. Kofoid and Swezy (1915) proposed less rigid host-specificity for T. augusta even earlier than Lavier. They found this species in the genera Rana, Hyla, Bufo, and Diemyctylus. Wood (1935) stated that he found T. augusta in Xantusia vigilis. Honigberg (1950) reported the trichomonad in other species of lizards.

Even though Alexeieff (1911) separated Tritrichomonas augusta from Tritrichomonas batrachorum, investigators still dealt with both species under Tritrichomonas batrachorum. Honigberg (1953) gave a description of both species. He states that T. augusta has a larger average size, and its body is typically more elongate. The

anterior flagella of T. batrachorum are definitely unequal, while those of T. augusta are about equal. In T. augusta the costa, unaccompanied by the paracostal granules, is stouter; also the undulating membrane shows typically more numerous and shallower undulations. The relatively slender axostyle of T. batrachorum, which contains no granules and is not surrounded by rings, projects for a considerable distance from the body, and its terminal part tapers gradually to a point. On the other hand, the robust axostyle of T. augusta shows axostylar granules, is surrounded by periaxostylar rings in its posterior part, and tapers rather abruptly immediately after leaving the body. The parabasal body, typically V-shaped in T. batrachorum, is rod- or sausage-shaped in T. augusta. The pelta is better developed in T. batrachorum.

Wenrich (1947) states that Tritrichomonas batrachorum from amphibian hosts established in culture is very adaptable, growing at room temperature in many different media. He states that much variation in longevity occurs among the strains of T. batrachorum from different frogs. The points illustrated by Wenrich's experiments are the varied survival potentialities for different species of flagellates from the same host grown in the same culture

tubes, the differences in survival times for the same species derived from different hosts, and the variations of the same strain in different culture media.

Bishop (1934) conducted experimental infections entailing Tritrichomonas batrachorum and Bufo vulgaris, Rana temporaria, and the larvae of Salamandra maculosa. She states that tadpoles of Rana temporaria can be infected with Tritrichomonas batrachorum isolated from Bufo vulgaris, and tadpoles of B. vulgaris can be infected with T. batrachorum from R. temporaria. She concludes, therefore, that T. batrachorum in frogs and toads is one valid species. She reports that cultures of T. batrachorum from B. vulgaris will infect larvae of Salamandra maculosa. Whittington (1951) transferred Tritrichomonas batrachorum from the intestine of the snake, Vipera ammodytes, to tadpoles. Cairns (1953) states that the parasitic-free condition of the tadpoles used in Whittington's transfaunation experiments is open to question. He quotes from Kessel (1930): ". . . the necessity for procuring absolutley parasite-free animals for experimental work cannot be too greatly emphasized." Cairns (1953) conducted transfaunation studies on the host-specificity of the enteric protozoa of amphibians and various other vertebrates. He

found that the species Tritrichomonas augusta and T. batrachorum appear to have morphologically and physiologically identical populations in North American amphibians. He reports that successful transfaunations may be expected when using these parasites with various combinations of North American amphibian donors and recipients. He states that Tritrichomonas batrachorum of snakes appears to be identical with T. batrachorum of Amphibia, and that T. batrachorum from Xenopus laevis, appears to be identical with T. batrachorum of North American Amphibia. Transfaunation between lizards and amphibians failed, as did those between amphibians and turtles. He concludes that host-specificity may not be rigidly determined and that species of intestinal protozoa should be based upon morphological distinctions supported, whenever possible, by experimental transfaunations.

Tritrichomonas batrachorum and T. augusta are widely distributed and apparently have little host specificity within the order Anura. As far as the author is aware, the present report is the first record of these species from Louisiana Anura.

Tritrichomonas batrachorum and T. augusta are briefly redescribed.

Trepomonas agilis Dujardin, 1841

(Plate IX, Figs. 50-54)

Host: *Rana catesbeiana*, *Rana clamitans*, *Rana pipiens sphenocephala*, *Pseudacris nigrita*, *Acris gryllus crepitans*, *Hyla crucifer crucifer*, *Hyla cinerea*, *Hyla avivoca*

Description: The species was found in the rectal contents of the hosts listed above. The hosts were collected in the vicinity of Baton Rouge, Denham Springs, Albany, Clinton, Amite, and Covington, Louisiana.

The description of this species is based on 40 specimens taken at random from eight different populations and is based on preparations stained and impregnated with iron-haematoxylin and silver-protein, respectively. The organism was rarely found and was found sparse in each of the host-species examined. This species was never recognized in the living state but was detected on slides prepared for the study of other protozoa.

Iron-haematoxylin and silver-protein preparations show the general outline of the organism as pyriform. The greatest width is in the middle third of the organism. Size range from eight different populations gave a range of from 10 to 12 microns in length by 4 to 7 microns in breadth. This is of interest, for different populations

of the same species from different host-species may differ significantly in this respect.

The organism possesses eight flagella having their origin in two groups of four, one group on either side of the body. Iron-haematoxylin-stained specimens do not readily show the flagella; however, silver-protein-impregnated specimens show each group consisting of a conspicuously long flagellum directed outward and reaching a length equal to that of the body and of three very short flagella. Because of the minute size of the flagella and their tendency to entwine with one another, it is not always possible to distinguish their actual number. Each group appears to have its origin from a basal granule complex consisting of two granules in a depression at the lateral border of the organism just behind the most posterior portion of the nuclei. The long flagellum originates from the most anterior of the two basal granules, whereas the short flagella appear to arise from the posterior basal granule. At times the short flagella lie in a depression or groove at the lateral borders of the organism (Figs. 50, 52). In some specimens the two groups of the short flagella can be seen to extrude from these depressions (Figs. 53, 54).

A distinguishing characteristic of the genus is the two nuclei. Iron-haematoxylin-stained specimens show each as a sickle-shaped structure at the antero-lateral portion of the organism. Together they appear to form a horse-shoe shaped structure with the apex situated at the extreme anterior end of the organism (Figs. 53, 54). The nuclear membrane is prominent, having chromatin granules adhering closely to the inner margin (Figs. 51, 53, 54). Silver-protein-impregnated specimens show the areas of the nuclei as finely granular structures.

At each lateral border of the organism is noted a cleft or depression. In some specimens these appear as narrow slit-like structures (Fig. 54); in others they assume a distended and gaping character (Fig. 52); in still other individuals this characteristic is obscured or lacking (Figs. 50, 51, 53). In some individuals the lateral margins of these structures are folded over the organism so that they appear as flap-like structures covering the posterior portion of the organism. The medial borders of the clefts stain deeply with iron-haematoxylin so that they appear to be supported by relatively thick fibers which terminate at the posterior lateral margin of the organism (Fig. 53). At times these borders

exhibit several very conspicuous undulations.

Iron-haematoxylin-stained specimens show the cytoplasm as vacuolated. Silver-protein-impregnated individuals assume a granular appearance. The presence of inclusions in the cytoplasm is noted (Fig. 50).

Comments: This genus has been recorded very few times. The present study showed no distinct morphological differences between the form described here as Trepomonas agilis and those forms described by Bishop (1937) from a pond at Shelford, England, as Trepomonas agilis.

According to Bishop, the species was first described by Dujardin (1841). Wenyon and Broughton-Alcock (1924) found a member of this genus in a mucous stool from a human being suffering from inflammation of the colon. Alexeieff (1909) states that he found the species in the intestinal tract of Amphibia, and in 1910 stated that the posterior portion of the intestine of Box salpa, a marine fish, was infested by a member of the genus. Lavier (1936) found Trepomonas agilis in the intestinal contents of Triton and in the tadpoles of Rana temporaria, Rana esculenta, and Alytes obstetricans. Das Gupta (1935) described the organism from Terrapene major, Kinosternon hippocrepis,

and Chelydra serpentina. Bishop (1937) describes its method of division.

This is the first report of the species from Anura of Louisiana.

Urophagus intestinalis (?) (Alexeieff) 1910

(Plate X, Figs. 55-59)

Host: *Rana catesbeiana*, *Rana clamitans*, *Rana pipiens*, *Microhyla carolinensis*, *Bufo terrestris*

Description: This species was found in the rectal contents of the hosts listed above. The hosts were collected in the vicinity of Carville, Baton Rouge, Denham Springs, and Amite, Louisiana.

Measurements of 50 specimens from five different populations give a range in length from 8 to 13.5 microns and in breadth from 4 to 7 microns. The general outline of fixed and stained specimens made with both iron-haematoxylin and silver-protein methods is pyriform. The anterior end is broadly rounded. The organism tapers more or less toward the posterior end; however, in some instances the posterior portion is so highly metabolic in nature that it assumes various morphological characteristics. The species possesses eight flagella, six anterior and two posterior. The anterior flagella appear to arise at the anterior end from the area in front of the two nuclei. Iron-haematoxylin-stained specimens show this area to be occupied by a basal granule complex which consists of two groups of granules separated from each other.

It is difficult to discern the number of granules in each complex. On the other hand, silver-protein-impregnated individuals show the anterior flagella arising from an area situated anterior to the nuclei with the base of each anterior flagellum composed of a short, rod-like structure. Close examination of these rod-like structures shows them as separate entities and as occurring in two groups of three each at the antero-lateral aspects of the nuclei (Figs. 57, 59). The distal portion of each rod gives rise to an anterior flagellum. The six anterior flagella are as long as or longer than the body of the organism.

The two axostyles appear to have their origin from the rod-like structures at the anterior end of the organism which give rise to the flagella (Figs. 56, 57, 59). They proceed in a posterior direction through the cytosome for a distance of approximately three-fourths to five-sixths the length of the organism, and then proceed to flare out as funnel-shaped developments, giving the impression that they are cytostomal openings (Figs. 55, 56, 58).

The origins of the two caudal flagella are vague, for the axostyles obscure this particular morphological characteristic. It is possible that they may have their origins at the same general site as do the anterior flagella.

The nuclei are circular to ovoid and lie at the extreme anterior end of the body (Figs. 55, 56, 57). Specimens impregnated with silver-protein show the nuclei as relatively clear areas (Figs. 56, 57, 59).

The cytoplasm of the organism is granular in nature.

Comments: When Moroff (1904) described a species of Hexamita from the rainbow trout, he used the generic name Urophagus; however, he regarded the species as identical with that of Hexamita intestinalis described by Dujardin (1841). When Dujardin (1841) erected the genus Hexamita, he described these flagellates as pear-shaped bodies with four anterior and two posterior flagella; hence, the genus was based on the presence of six flagella. According to Dobell (1909), the change is unwarranted because Urophagus was established by Klebs (1892) and contained a single species, which differed from all other six-flagellate organisms in the respect that it ingested food at the posterior end of the body. Moroff never observed this phenomenon. Klebs defines the genus Urophagus as follows: ". . . Körper eiformig bis schmal langlich, hinten schnabelformig zugespitzt. An der Seite des Schnabels je eine schmale langliche Spalte, in der die zwei Schleppgeisseln sitzen; vorn zwei Paare von drei Geisseln. Der Schnabel

besteht aus zwei beweglichen Klappen, mit denen feste Nahrung aufgenommen wird. Kontraktile Vacuole in der Mehrzahl an den Seitenrandern."

Alexeieff (1910) described, and depicted a form in Motella tricirrata and M. musteta. This form he evidently regarded as identical with Urophagus intestinalis Moroff (1904). However, Moroff observed an organism which he designated as U. intestinalis in the living state. Moroff's figures and description are incomplete. It is difficult to know exactly what he was observing. Alexeieff (1910) redescribed and depicted a species which he states is identifical to that of U. intestinalis. Alexeieff's figures and description do not bear any similarity to that of Urophagus intestinalis Moroff (1904).

The form observed by the present author in the anuran hosts examined have morphological characteristics remarkably similar to that of Urophagus intestinalis (Alexeieff) 1910. Alexeieff depicts the twin axostyles as widely separated from one another and having a funnel-shaped flare-out at the posterior ends of these structures. These funnel-like expansions are located in the anterior portion of the most posterior section of the body proper. He depicts the twin nuclei as rounded or ovoidal and located

at the most anterior end of the organism. Six anterior flagella arise from a fused blepharoplastic-complex, which in turn is located in front of and medially to the twin nuclei. Moroff (1904) states that the size of his species is 12 to 16 microns in length and 6 to 7 microns in breadth. Alexeieff indicates the size of his species as approximately the same as that of Moroff's specimens.

Examination of the species in the current study shows morphological similarities to that of Alexeieff's species, as was noted above; therefore, the author is reluctant to designate a new species for those forms observed in the present study. He tentatively designates the form observed here as Urophagus intestinalis (?) (Alexeieff) 1910.

In this study Urophagus intestinalis (?) (Alexeieff) 1910 is redescribed. Specimens impregnated with silver-protein are described. This is the first report of U. intestinalis from Anura of Louisiana.

Hexamitus intestinalis Dujardin, 1841

(Plate XI, Figs. 60-64)

Host: *Rana areolata*, *Rana catesbeiana*, *Rana clamitans*, *Rana grylio*, *Rana pipiens*, *Hyla cinerea*, *Hyla crucifer*, *Hyla avivoca*, *Hyla gratiosa*, *Pseudacris nigrita*, *Microhyla carolinensis*, *Acris gryllus crepitans*, *Bufo terrestris*, *Bufo fowleri*, *Bufo valliceps*

Description: *Hexamitus intestinalis* is a common intestinal flagellate of the hosts listed above. This flagellate was collected from the rectum of the hosts mentioned. The hosts infected were collected from Pineville, Leesville, De Ridder Airbase, Baton Rouge, Carville, Denham Springs, French Settlement, Springfield, Albany, Pine Grove, Mandeville, Covington, Pearl River, Louisiana.

Measurements of 100 specimens from 10 different populations give a range in length from 6 to 11.5 microns, with an average of 9.5 microns, and a width range from 2 to 6 microns with an average of 3.5 microns.

The general outline of fixed and stained specimens made with both iron-haematoxylin and silver-protein methods is ovoidal to pyriform. The anterior end may be broadly rounded; however, in some instances it is bluntly pointed.

Hexamitus intestinalis possesses eight flagella, six anterior and two posterior. The six anterior flagella

arise at the anterior end in front of the nuclei. In some instances they arise between the two nuclei. Iron-haematoxylin-stained specimens show this area to be occupied by three to six small basal granules, the two medial granules, as often as not, being fused. Four of the six anterior flagella appear to arise from the fused complex, whereas the other two anterior flagella arise from each of the two laterally-situated basal granules (Fig. 60). Silver-protein-impregnated individuals show the six anterior flagella arising from an area situated anterior to the nuclei. The bases of the anterior flagella are composed of rod-like structures which impregnate intensely with silver-protein. The rod-like structures have their origin from an area just anterior to the two nuclei. Close examination of these structures show them separated into two groups, which appear as inverted Y-shaped constructions. The distal portion of each arm of these structures gives origin to the anterior flagella. All six anterior flagella are as long as or longer than the body.

The organism possesses two distinct axostyles which appear to arise from the anterior end of the body. It is difficult to discern the origin of the axostyles in iron-haematoxylin specimens (Figs. 60, 61); however, silver-protein-impregnated individuals show their origin from

the center of each inverted Y-group (Fig. 63). They proceed in a posterior direction, at first just medial to the most internally placed arm of the Y, then curve laterally from the longitudinal exis of the organism, to terminate at the posterior end of the body. In many specimens the axostyles bend backward toward each other, and appear to cross each other (Fig. 64). The posterior end of each axostyle flares out slightly and terminates flush with the posterior portion of the body.

The caudal flagella appear to arise from an area at the posterior portion of each axostyle; however, this morphological trait is not clear in either iron-haematoxylin or silver-protein specimens. It is quite possible that they may have their origin from the anterior end at the region of the Y-groups as do the anterior flagella.

The organism possesses two nuclei which are the most characteristic structures of this species. The two are situated at the anterior end immediately lateral and slightly behind the basal granule complex. The iron-haematoxylin-stained specimens these nuclei appear as large club-shaped chromatin bodies having a length of from 2 to 6 microns. As often as not the two nuclei are fused at the anterio-medial portion so that the entire structure,

consisting of both nuclei, takes on the appearance similar to that of a horseshoe. At times the medial portions of the nuclei cross each other (Fig. 62). Silver-protein-impregnated specimens show these nuclei as elongated cloud-like structures speckled with lightly-staining minute bodies. In many specimens the nuclei were not impregnated, and the area in which they are located appeared as a clear, elongated zone (Figs. 62, 64).

The cytoplasm appears reticulated in iron-haematoxylin, whereas silver-protein shows the cytoplasm to be granular.

Comments: Dujardin (1841) erected the genus Hexamita for a species found in the frog and for two others which were free living organisms. He gave the name Hexamita intestinalis to the parasitic form and designated the free-living forms as H. nodulosa and H. inflata. Bütschli (1878) modified the generic name of Dujardin to Hexamitus. Kirby and Honigberg (1949) state that Hexamitus is more proper etymologically. They state that under the provision of Article 19 of the International Rules of Zoological Nomenclature, the correction is in order.

In this study, the forms that had been stained in iron-haematoxylin agree in essential morphological details

to those described by Swezy (1915) as Hexamitus intestinalis and H. ovatus from Diemyctylus torosus, Aneides lugubris, Plethodon oregonensis, Batracoseps attenuatus, Rana boylei, R. draytoni, and R. pipiens, and to the species described by Lavier (1936) as Spironucleus elegans from amphibian hosts. Swezy described and depicted her species, H. intestinalis, as ovoidal to pyriform, tapering more or less toward the posterior end. She states that the size of the organism varies from 9 to 12 microns in length by 5 to 8 microns in breadth, although forms both above and below these limits are encountered. She describes and depicts the twin nuclei as large, club-shaped masses from 3 to 5 microns in length. She states that the anterior portions of these nuclei are often fused with the basal granule complex, so that together the structures appear horseshoe-like. She further describes the two axostyles as lying parallel to each other and placed longitudinally in the cytoplasm. She describes the organism as possessing six anterior and two posterior flagella.

The form designated by Swezy as H. ovatus is described and depicted by her as ellipsoid to ovoid and measuring 6 to 8 microns in length. The nucleus is elongated and rounded at both ends and measures 1.5 by 2 or 3

microns in size. A very definite nuclear membrane is present, which she states is especially prominent prior to division. The axostyles are slender and run a longitudinal course through the body. They are often crossed over each other in the posterior portion of the body. The organism possesses six anterior and two posterior flagella.

Lavier (1936) depicted the species which he designated as Spironucleus elegans as fusiform. He stated that the nucleus is elongated and assumes a spiral shape and that the paired axostyles run longitudinally through the cytoplasm. He also states that his organism possesses six anterior and two posterior flagella.

The conditions and circumstances under which this species was found in the present study indicate that the flagellate given the name H. ovatus by Swezy (1915) and the one designated by Lavier (1936) as Spironucleus elegans are in actuality forms of H. intestinalis Dujardin (1841). The study revealed that as often as not all three forms appear to be present within different populations of the same host-species and within intra-populations of a particular host-species. The possibility exists that these types are only developmental forms of H. intestinalis. An investigation of the complete life-cycle of these

individual forms probably would establish the fact that they are of the same species. Swezy (1915) states that the form she designates as H. ovatus follows the same general process of binary and multiple fission as H. intestinalis. Her study of the living animals was made possible by sealing the cover glass with vaseline after adding a few drops of normal salt solution to the material from the intestine. She states that H. ovatus was found in abundance in only one host, Diemyctylus torosus, occurring only sparingly in the other amphibians examined.

From an overall picture, it was found that the various forms appeared to integrate with one another; that is, no sharp and abrupt demarcation occurred among them. The nuclear structures upon which H. ovatus and Spironucleus elegans are based depend on the stage of the organism and the position the nucleus was in, when it was fixed and stained. In addition, silver-protein-impregnated specimens show similar Y-shaped constructions at the site of the bases of the anterior flagella in all three forms. Positive identification can only be based on pure culture and life-history methods. An attempt to attach other generic and specific names to these forms leads to confusion in later work.

Although this species has been recorded from various

amphibian hosts, this is the first record of the parasite from Anura of Louisiana. Specimens stained with iron-haematoxylin and impregnated with silver-protein are described.

Octomastix batrachorum (Swezy) 1915

(Plate XII, Figs. 65-71)

Syn: Hexamitus batrachorum Swezy, 1915

Host: Rana areolata, Rana catesbeiana, Rana clamitans, Rana grylio, Rana pipiens, Hyla avivoca, Hyla crucifer, Pseudacris nigrita, Acris gryllus crepitans, Bufo terrestris, Bufo fowleri, Bufo valliceps

Description: This species is one of the more common intestinal flagellates found in this study. The flagellate was found in both the anterior and posterior portions of the large intestines.

The hosts infected with this species were collected in the vicinity of Leesville, De Ridder Airbase, Carville, Baton Rouge, Napoleonville, Denham Springs, Springfield, Pine Grove, Hammond-Pontchatoula, Covington, and Slidell, Louisiana.

Measurements of 100 specimens of this species from 10 different populations give a range in length from 4 to 6 microns, with an average of 5.5 microns, and a width range from 3 to 4 microns, with an average of 3.25 microns. The general outline of the organism is ellipsoidal, having both anterior and posterior ends rounded.

The organism possesses eight flagella, six anterior

and two posterior. Well differentiated iron-haematoxylin-stained specimens show six anterior flagella arising from four to six individual basal granules, which in turn have their origin at or near the anterior and lateral surfaces of the nuclear membranes (Figs. 65, 67). A few specimens show a fibril connecting each antero-medial basal granule to each of the postero-laterally placed basal granules (Fig. 66). There are instances where each of the postero-laterally placed granules are connected to each other by a connecting fibril (Fig. 66).

Silver-protein-impregnated individuals show six anterior flagella arising from six separate basal granules situated at the anterior and lateral aspects of the organism. Some silver-protein specimens show the individual basal granules connected to each other by a fine filament (Fig. 69). The bases of the flagella are composed of rod-like structures which impregnate with silver-protein (Figs. 69, 70, 71). The intensity of impregnation by the silver ion onto these structures is dependent on the pH of the silver-protein medium.

The origin of the two posterior flagella is not clear, for the axostyles of the organism make it difficult to follow this particular morphological characteristic. It

is quite possible that they have their origin, as do the anterior flagella, from the basal granule complex. All eight flagella are as long as or longer than the length of the body.

The organism possesses two slender axostyles. Silver-protein-impregnated specimens show each axostyle arising from individual basal granules of the most posterior group, whereas in iron-haematoxylin specimens it is difficult to discern where they arise, for the nuclei obscure their true origin. The axostyles pass posteriorly through the cytoplasm and terminate flush with the posterior border. The width of the axostyles in both iron-haematoxylin and silver-protein specimens does not vary significantly.

The nuclei are relatively large, circular to ovoid structures which lie at the extreme anterior end of the body. At times they appear fused together at their medial borders. The nuclei stain intensely black with iron-haematoxylin. Well-differentiated specimens show chromatin granules at the peripheral region of the nuclei. In some individuals a minute endosome can be observed which lies near the center of the nucleus (Fig. 67). Specimens impregnated with silver-protein show the nuclear area as a finely granular structure, making it possible to observe the true origin of the axostyles and the anterior flagella (Figs. 70,

71). At times, silver-protein specimens show the nuclei as spherical structures, at the center of which can be seen the minute endosomes.

One of the most distinguishing characteristics of the species is the two large extranuclear chromidial bodies situated at the posterior end of the organism and generally occupying a position between and near the slender axostyles. These bodies stain intensely black in iron-haematoxylin specimens and are usually spherical in structure (Fig. 66). In some instances the spherical structures are surrounded by a halo-like area (Fig. 65). Some of these bodies appear to be situated on the posterior portion of the axostyles. Silver-protein-impregnated specimens show these structures as hollow spherical to ovoidal doughnut-like rings; in many instances they assume ellipsoidal, hollow shapes becoming egg-like in appearance (Figs. 68, 70, 71). These structures lie free in the cytoplasm; no connecting fibril is detectable at any time connecting the structures to any other structure within the cytoplasm. Silver-protein impregnates these bodies bluish-black in media having a pH of 6.8 to 7.2; they assume, however, a pinkish hue at a pH of 7.8 to 8.5. These structures disappear completely at a pH of 6 and below and at a pH of 8.5

and above. At times they appear fused to one another, or may assume different positions in relation to each other at the posterior end of the organism (Figs. 68, 70, 71).

Iron-haematoxylin shows the cytoplasm as reticulated. Silver-protein, on the other hand, shows it as granular.

Comments: This species was originally described by Swezy (1915) as Hexamitus batrachorum from Rana pipiens, Batracoseps attenuatus, and other amphibians examined by her. She states that it resembles in its nuclear structure the Hexamitus depicted by Alexeieff (1912) from Nicoria trijuga, from Ceylon, which he named Hexamitus parvus. His figures, however, are not supplemented by a description. Alexeieff (1917) then placed the form seen by him (1912) in a new genus, Octomastix. Grassé (1924), who studied Octomastix parvus in the urinary bladder of Emys orbicularis, accepts this genus by stating, "Les caractères morphologiques d'O. parvus justifient la creation, par Alexeieff du genre Octomastix, qui s'apparente d'ailleurs au genre Hexamitus. Nous le définirons: Deplozoaire parasite à noyaux tres anterieurs et ovoides à chromatine granuleuse; blépharoplastes antérieurs placés latéralement par rapport aux noyaux, unis aux postérieurs par une desmose périnucléaire. Flagelles antérieurs s'inserant directement sur les

blépharoplastes. Rhizostyles courts et arqués."

Swezy (1915) states that Alexeieff's O. parvus shows distinctive characteristics which separate it from the flagellate described by her as H. batrachorum. The characteristics which separate it from H. batrachorum, as stated by her, are (1) the point of origin of the flagella, which figures in O. parvus arise laterally in two groups, widely separated from one another, while in H. batrachorum they are anterior and closely connected; (2) the extranuclear chromidial bodies are of a definite shape and position in both forms. In O. parvus they have a circular form and occupy an anterior position between the axostyles, while in her species, H. batrachorum, she states that they are situated on the axostyles near their posterior extremities.

From the study of abundant material in the writer's collection, certain evidence was presented which indicated that a restudy of H. batrachorum should be made. In H. batrachorum, Swezy (1915) described the three pairs of anterior flagella as arising from two basal granules which are often massed together indistinguishably. These rest upon the surface of the nuclei. This morphological characteristic is depicted in the one and only drawing by her of the non-dividing form. In a restudy of this characteristic,

based on iron-haematoxylin-stained specimens and taken from the collection of the author, it was found that in many instances the blepharoplastic complex appeared to be massed together indistinguishably, so that the complex very often appeared to consist of two large basal granules situated at the anterior end of the organism. Close examination on slides critically differentiated showed the complex consisting of from four to six basal granules which were widely separated from one another. In addition, silver-protein-impregnated specimens clearly bring out the existence of six basal granules situated at the antero-lateral aspects of the organisms. They are situated lateral to the nuclei and are relatively widely separated from one another. It is possible that Swezy missed the true count of the basal granules in her description of the species, for it is difficult to discern the true number of basal granules stained with iron-haematoxylin because of the obscuring effect of the nuclei.

The presence of the extranuclear bodies situated on the axostyles and near the posterior extremities of these structures, as depicted by Swezy (1915), was found by the author to occupy, in reality, a position between the axostyles; however, when viewed at a certain angle, these

structures appear to occupy a position on the posterior portion of the axostyles. In addition, she states that they are of a definite shape and occupy a definite position. The author found that these structures may assume different positions in relation to each other at the posterior end of the body, and that various sizes within different individuals are noted. From the one drawing by Swezy it appears that she may have misinterpreted the position of these structures or did not examine sufficient numbers of individuals to verify the true relationship of these structures to the axostylar elements and to the body of the organism. The use of silver-protein clearly brings out the relationship of these structures to the other elements within the cytoplasm.

Examination of the O. batrachorum taken from Anura, leaves little doubt that Hexamitus and Octomastix are distinct genera. The author does not agree with Lavier (1936) that Octomastix is a synonym of Hexamitus. It was found that this species has the generic characteristics specified by Grassé (1917) and therefore should be placed in the genus Octomastix rather than retain it in the genus Hexamitus. Wenrich (in a personal communication) concurs with this designation.

This is the first report of O. batrachorum from

Anura of Louisiana. In addition, the species is redescribed and specimens impregnated with silver-protein are described. Octomastix batrachorum possesses certain morphological characteristics revealed for the first time by the silver-protein technique.

Octomitus neglecta (Lavier) 1936

(Plate XIII, Figs. 72, 73, 75)

Syn: Syndyomita neglecta Lavier, 1936

Host: Rana areolata, Rana catesbeiana, Rana clamitans, Rana grylio, Rana pipiens, Rana palustris, Hyla avivoca, Hyla crucifer, Pseudacris nigrita, Acris gryllus crepitans, Bufo terrestris, Bufo fowleri, Bufo valliceps, Microhyla carolinensis

Description: This species of intestinal flagellate was found in the rectal contents of the hosts listed above. The hosts were collected in the vicinity of Farmerville, Sterlington, Carville, 20 miles S. S. E. Baton Rouge, Thibodaux, Denham Springs, French Settlement, Springfield, Albany, Amite, Covington, Slidell, Pearl River, and Bogalusa, Louisiana.

Observations reported here by the author are based on 100 specimens taken at random from 10 different populations. Silver-protein impregnation has not been utilized in studies of Octomitus from Amphibia; therefore, this method has revealed morphological features which are of great interest.

Individuals of the species show no significant variation in shape, although the size of the individual does vary. The body of the flagellate appears pyriform with the

anterior end broadly rounded. The flagellate measures from 6 to 11.5 microns in length by 4 to 8.25 microns in breadth. Measurements are based on individuals with comparatively straight axostyles.

The flagellate possesses six anterior flagella and two posterior flagella. The anterior flagella are directed backward in both living and fixed specimens. Iron-haematoxylin specimens show each anterior flagellum originating from separate basal granules which are situated at the extreme anterior end of the organism (Fig. 72). Silver-protein-impregnated specimens show these flagella arising from the distal ends of six rod-like silver-protein impregnating bodies which in turn have their origin from the area between the two nuclei (Fig. 75). Close examination of these structures show that the rods are separated into two groups, appearing as Y-shaped arrangements when viewed from the anterior end (Fig. 75). The rods measure approximately 2 to 2.25 microns in length. From the distal end of each of the extremities of the "Y," the flagella continue free (Figs. 73, 75). The caudal flagella appear to have their origin from an area at the posterior end of the split axostylar structure, although it is possible that they may arise from the "Y" at the anterior end of the organism. This

morphological characteristic is not clear (Figs. 73, 75). Near the posterior end of the cytosome they pass out of the body, becoming free. They are generally as long as the body, but at times they appear to be longer than the body.

The axostyles appear as a fused structure with both anterior and posterior ends fractured. Each of the two anterior ends appears to have its origin from the center of each "Y" group (Fig. 75). The posterior ends of the fused axostylar structure flare out and terminate flush with the posterior portion of the cytoplasm (Fig. 75).

The two nuclei are comparatively small, circular to ovoid structures which lie at the extreme anterior end of the body. Iron-haematoxylin reveals, as often as not, two round endosomes which lie at the center of the nucleus (Fig. 72). Silver-protein reveals the nucleus as a cloud-like ovoid structure, devoid of endosomes (Fig. 75).

The cytoplasm appears reticulated in iron-haematoxylin-stained specimens, whereas the silver-protein technique shows it as granular.

Comments: Iron-haematoxylin-stained specimens reveal this flagellate as Lavier's (1936) Syndyomita neglecta, which he found in amphibian hosts. He specified that the generic characteristics of this flagellate are based on the

fact that the axostylar structures related to the caudal flagella are close together and parallel, adherent, or fused in the medial longitudinal exis of the body. He did state, though, that the generic characteristic of this flagellate is present in those of the rat which Prowazek (1904) described as Octomitus intestinalis. He stated that chaos could be eluded by avoiding Prowazek's generic name for it, inasmuch as this had not been distinct and had been adopted with lack of discrimination to flagellates of the Hexamitus type. Kirby and Honigberg (1949) state that if there is to be a generic distinction within the Hexamitus group of flagellates, Octomitus with type intestinalis has priority over Syndyomita with type neglecta, if the rat and amphibian flagellates have the same generic characteristics.

The description of iron-haematoxylin specimens agrees in all essential details to that given by Lavier (1936). In addition, specimens impregnated with silver-protein are described. Octomitus neglecta possesses certain morphological features revealed by silver-protein impregnation that have not been detected before in the species. These structures have been discussed above.

Although this species has been recorded from various amphibian hosts, this is the first report to record the parasite from Anura of Louisiana.

Trimitus parvus Grassé, 1932

(Plate XIII, Figs. 74, 76)

Host: Rana catesbeiana, Rana clamitans, Rana pipiens sphenocephala, Bufo fowleri, Bufo valliceps

Description: The form designated as Trimitus parvus is one of the rare intestinal flagellates of the hosts listed above. The flagellate was found in smears made from the rectal contents of the hosts. The host-species were collected in the vicinity of Baton Rouge, Denham Springs, Amite, and Carville, Louisiana.

The description of this species is based on 30 specimens taken at random from five different populations and is based on preparations stained and impregnated with iron-haematoxylin and silver-protein. The organism was rarely found and was found sparse in each of the host species examined. This species was never recognized in the living state but was detected on slides prepared for the study of other protozoa.

In size, the flagellate ranges from 3 to 7 microns in length by 2 to 5 microns in breadth. It is ovoid to pyriform with the anterior broader than the posterior end. in some specimens the posterior end terminates in a point.

The organism possesses two anterior flagella of unequal length which have their origins at or near the surface of the nucleus (Figs. 74, 76). The longer one has a length equal to that of the body, whereas the shorter one is less than the length of the organism. In addition, the organism possesses a posteriorly-directed flagellum, which appears to originate near or at the point of origin of the anterior flagella. It extends the length of the body posteriorly, adheres closely to the cytoplasm, and then becomes free to trail behind the body. It is approximately twice the length of the body.

The nucleus is ovoid. An endosome was not seen in any of the specimens studied. Originating near the nucleus and running longitudianlly and parallel to the recurrent flagellum is a thin chromatin-like fibril which extends posteriorly in the cytoplasm for two-thirds the length of the body (Fig. 74). It is possible that this structure may be analogous to the structure which Nie (1950) called the funis in Enteromonas.

Iron-haematoxylin and silver-protein-treated specimens failed to reveal either axostyles or parabasal bodies.

Iron-haematoxylin-stained specimens show the cytoplasm as vacuolated; however, silver-protein-impregnated

individuals show it to be granular.

Comments: The forms described in this study that have been stained and impregnated with iron-haematoxylin and silver-protein agree in essential morphological detail to those forms described by Saxe and Schmidt (1953) and found by them in Thamnophis radix and Rana pipiens. The only apparent difference is that forms from the author's collection average slightly larger than those described by Saxe and Schmidt. There are not sufficient differences for regarding the form found in this study as a distinctly different species.

Alexeieff (1910), utilizing iron-haematoxylin, depicted from Onos tricirrata, an intestinal flagellate which he named Trimitus motellae. He stated that the flagella characterized this organism. He described two anterior flagella of unequal length, one shorter, the other longer than the body. In addition, Alexeieff noted a long recurrent flagellum, up to four times the body length, joined in its proximal portion to the surface of the body of the organism. He showed the nucleus as occupying a region in the extreme anterior portion of the body. The size of the organism was designated as 6 to 8 microns in length.

Grassé (1932) described Trimitus parvus from Rana

temporaria and Bufo vulgaris. The organism is described as having two anterior flagella, one shorter, the other of equal length or longer than the body of the organism. He also described, in addition to this, a rod-shaped parabasal body and a lightly-staining filiform structure which he designated as an axostyle.

Wenrich (1945) identified Trimitus parvus from Rana pipiens. In addition, Knowles and Das Gupta (1930) reported Trimitus sp. from a turtle.

Saxe and Schmidt (1953) reported Trimitus parvus from Rana pipiens and Thamnophis radix and confirmed the generic characteristics given by Alexeieff (1910). Their observations, utilizing silver-protein, did not reveal a structure identifiable as a parabasal body. In addition, these investigators failed to find an axostyle. They reported the presence of a funis in the species and considered that the presence of this structure is evidence of a relationship to Enteromonas.

From the study of the material in the writer's collection, evidence is presented and is correlated with Saxe and Schmidt's studies, which indicate that Trimitus parvus lacks an axostyle and parabasal body. It was found that Bouin's solution, without 5 per cent acetic acid, failed to

bring out a parabasal body. In addition, the structure was not demonstrable with silver-protein. Smears on which this species was found and that had been impregnated with silver-protein contained the species, Tritrichomonas augusta. The parabasal body of Tritrichomonas augusta was clearly brought out; therefore,this fact is strong evidence that Trimitus parvus lacks a parabasal body.

As indicated by Saxe and Schmidt, the genera Trimitus and Enteromonas differ significantly only in the number of anterior flagella. Trimitus possesses two, whereas Enteromonas has three. They state that if Trimitus lacks an axostyle and parabasal body, the genus should be removed from the Trichomonadida where it was placed by Grasse (1952) and placed with Enteromonas in the Cercomonadidae as Kirby (1930) suggested.

This is the first report of the species from Anura of Louisiana. Rana catesbeiana, Rana clamitans, Bufo valliceps, and Bufo fowleri constitute new records as host-species for this intestinal flagellate.

GENERAL DISCUSSION OF HOST-PARASITE RELATIONSHIP

The Anura possess a considerable degree of general similarity yet show a range of habitats and habits which profoundly influence their parasitic faunas.

The Anura of Louisiana provide excellent material for the study of ecological problems related to parasitism. They live in habitats ranging from aquatic to those which are more or less completely terrestrial and/or arboreal.

The mild climate permits many species of host to remain active for the greater part of the year. Opportunity is offered to make interesting comparisons of the effect of habitat on haemoflagellates and intestinal flagellates. In addition, seasonal variations, host specificity, multiple infestations, and other ecological phenomena may be studied.

The flagellates studied belong to two distinct biological groups--the haemoflagellates and the intestinal flagellates.

The study shows that anurans of Louisiana are parasitized by trypanosomes. Trypanosomes are more often found

in the aquatic hosts, which suggests an intermediary aquatic blood-sucking vector.

The genera Bufo and Scaphiopus were found free of infection. This may be correlated with the absence of leeches (the intermediate hosts), the infrequency of the individual anuran to reside in the aquatic environment, or a certain degree of immunity.

It is known that the class Hirudinea is the intermediate vector for the transmission of amphibian trypanosomes. Franca (1908) and Nöller (1913) demonstrated that the leech, Hemiclepsis marginata, is the intermediate host of T. rotatorium. They demonstrated that two or three days after feeding on an infected tadpole, leptomonod forms were present in the stomach of the leech. Then, at the end of a week, slender trypanosomes occurred and these gradually replaced the leptomonad forms. Toward the end of digestion these trypanosomes migrated forward to the proboscis, and passed out of the oral cavity into the proboscis sheath, where they multiplied profusely. Infection took place from the proboscis sheath during feeding. Nöller stated that after feeding, the leech emptied its proboscis sheath and reproduction of the trypanosomes began again in the stomach, and that reinfection of the proboscis sheath occurred again at the end of digestion. He stated that development of the

trypanosomes in the leech took place in the stomach.

In general, the more aquatic frogs, such as those of the genus Rana, have a longer larval period than the more terrestrial and arboreal species. Within this genus, the percentage of infection was remarkedly higher than in any other. The arboreal anuran hosts harbored trypanosomes; however, the incidence was very low. The author believes that there are several reasons why this is true. In the first place, the larval stage of tree frogs is relatively short. In addition, after reaching maturity they reside in bushes and trees away from water and usually approach water for spawning activity only.

Nöller (1913) showed that infection in adult frogs (R. esculenta) may be superimposed through inoculation with infected blood from tadpoles of the host-species, or by infection with large doses of cultured trypanosomes.

Examination showed that within species of the genus Rana harboring trypanosomes there appeared no significant seasonal variation. The trypanosomes were as prevalent during the colder periods of the year as in the warmer periods. There may be three reasons for this. First, the infected vector may feed during the entire year and thereby may infect the anuran host at any time. Second, a superim-

posed infection could occur in this manner. Third, the anuran hosts could possibly retain their infection through long periods of time.

Nöller showed that T. rotatorium of the frog exhibits a great variety of shapes and forms; and although a mixed infection of two or more species is possible, it is safer to designate the different morphological forms found in a given individual as cyclic phases of T. rotatorium. In view of their highly polymorphic nature, the various morphological forms of anuran trypanosomes can only be proven as distinct species by applying serological tests and by cultivation in differential media.

Nigrelli (1945) suggests that a simple experiment which would add much to our knowledge of the trypanosomes of Anura would be to test for the trypanolytic action of sera from various amphibians on cultured strains of a known species. The author concurs with Nigrelli's suggestion.

There are very few records concerning the effect of trypanosomes on anuran hosts. Nöller (1913, 1917) published accounts of inoculation experiments performed with the trypanosomes of frogs. The blood of tadpoles of Rana esculenta infected with trypanosomes was inoculated into adult frogs, which developed a larger infection of the

forms characteristic of frogs than they had before. He carried out inoculations with large doses of cultured forms from blood-agar plates. Though the frogs already had a small infection, they developed an enormous one, which killed them. Nöller further states that the blood and organs were swarming with large trypanosomes. These infections could possibly have been superimposed on former infections.

The author never observed ill effects produced by trypanosomal infections.

The general method by which intestinal flagellates may infest the anuran host is by the ingestion of cysts and resistant trophozoites from contaminated aquatic environment in which the host resides. Presumedly, the fecal material of infected hosts residing in water contaminates the water and food in the given areas and are ingested by other Anura.

Bishop (1934) showed that the survival periods for T. batrachorum in sterile rain water ranged from 48 hours to five months. Rosenberg (1936) stated that T. augusta survived for 347 days in 0.85 per cent NaCl solution with a pH of approximately 7.55. Wenrich (1947) observed that T. batrachorum lived from 1405 to 1475 days in cultures

made originally in pond water. The observations of these investigators indicate that the aquatic spawning areas of Anura may provide a perpetual source of protozoan parasites.

As pointed out by Cairns (1953), "Host specificity may depend on the opportunities of the parasite to infect potential hosts and its ability to survive all transitional stages (digestive juices, drying, etc.) which might be encountered during the passage from one organism to another. Therefore, a particular parasite which may be able to exist in a certain area of a potential host-species may not be found there because of lack of opportunity or because of its inability to survive one or more phases of the journey between hosts. Because these aspects of host-specificity are subject to variation and change they should be given consideration."

As stated by Cairns (1953) "Two schools of thought regarding host-specificity are extant. The first school regards host-parasite relationships as rigid. They use this relationship as a basis for species separation. The other school states that morphologically similar parasites which are found in different hosts may belong to a single species."

Kessel (1930) states that "each species of host and of parasite must be considered on the basis of its own individual relationships."

Becker (1933) states that "host-specificity is the peculiar adaptation of a species to the environment within or on another species or more or less limited groups of species." He points out that a parasite shows rigid host-specificity or loose host-specificity according to whether it confines or fails to confine its host selection to a certain restricted group of animals.

Wenrich (1935) found in his studies of host-parasite relationships that the genus Hexamitus is of great interest. He states, "If we try to select the Hexamita species that are most alike morphologically, we find that they have no parallel relation to the taxonomic relationships of the hosts." He states that "there is almost as much morphological variation among the free-living freshwater species as there is among the parasitic ones." Among the parasitic species he found several distinctive types of morphology in the same species of frogs; for example, in Rana pipiens, and even in the same individual frog at different times. He states that the species from the monkey most resemble certain ones from frogs and turtles. He also specifies that there is one species in rats and mice in the caecum and another in the small intestine. The one in the caecum, instead of showing resemblance to the one in the small intestine of the same host, more nearly resembles certain

species in the frogs, toads, and salamanders. He also states that a species found on the outside of a marine fish more nearly resembles one from the milliped than it does one from the inside of another marine fish. He states that "Evidently there is little parallelism between the morphology of the endozoic species of the Hexamita and the taxonomy of their hosts." Transfaunation experiments and serological tests may give evidence in support of the validity of the described species of intestinal parasites.

The data presented in this study are not intended to be conclusive regarding the incidence of parasitism of haemoflagellates and intestinal flagellates in Louisiana Anura. The author has scratched the surface of a field of research which to this date is almost untouched. Certainly it is the initial point for future observation and investigation on the protozoan fauna of the Anura of Louisiana.

The numbers of different species of Anura examined are unequal. In addition, every area of Louisiana was not covered. This should be considered and kept in mind before attempting to interpret the list of hosts and their flagellate parasites.

The following list shows the frequency of occurrence of the protozoan parasites in the host-species.

TABLE IV

CHECK LIST OF THE HAEMOFLAGELLATES AND INTESTINAL FLAGELLATES OF LOUISIANA ANURA

Species	Number Species Anura Infected
Trypanosoma karyozeukton	1
Trypanosoma rotatorium	10
Retortamonas dobelli	8
Chilomastix caulleryi	9
Karotomorpha swezyi.	5
Monocercomonoides elegans n. sp.	2
Monocercomonoides melolonthae	2
Monocercomonas batrachorum	8
Tritrichomonas augusta	18
Tritrichomonas batrachorum	14
Trepomonas agilis	8
Urophagus intestinalis (?)	5
Hexamitus intestinalis	15
Octomastix batrachorum	12
Octomitus neglecta	14
Trimitus parvus	5

TABLE V

HAEMOFLAGELLATES AND INTESTINAL FLAGELLATES OF LOUISIANA ANURA, LISTED AS TO HOSTS

PROTOZOAN	HOST-SPECIES											
	Rana areolata		Rana catesbeiana		Rana clamitans		Rana grylio		Rana palustris		Rana pipiens	
	Exam.	+	Exam.	+	Exam.	+	Exam	+	Exam.	+	Exam.	+
Trypanosoma karyozeukton	15	-	38	-	36	-	17	-	4	-	33	-
Trypanosoma rotatorium	15	2	38	11	36	9	17	2	4	1	33	12
Retortamonas dobelli	15	-	38	2	36	3	17	-	4	-	33	2
Chilomastix caulleryi	15	-	38	10	36	6	17	-	4	-	33	4
Karotomorpha swezyi	15	-	38	9	36	7	17	-	4	-	33	8
Monocercomonoides elegans n. sp.	15	-	38	-	36	-	17	-	4	-	33	-
Monocercomonoides melolonthae	15	-	38	-	36	-	17	-	4	-	33	-
Monocercomonas batrachorum	15	-	38	-	36	7	17	1	4	-	33	4
Tritrichomonas augusta	15	11	38	22	36	19	17	13	4	2	33	24
Tritrichomonas batrachorum	15	-	38	11	36	9	17	6	4	-	33	14
Trepomonas agilis	15	-	38	6	36	2	17	-	4	-	33	3
Urophagus intestinalis (?)	15	-	38	5	36	7	17	-	4	-	33	11
Hexamitus intestinalis	15	4	38	21	36	19	17	6	4	-	33	23
Octomastix batrachorum	15	8	38	18	36	14	17	5	4	-	33	24
Octomitus neglecta	15	2	38	6	36	18	17	3	4	1	33	19
Trimitus parvus	15	-	38	1	36	1	17	-	4	-	33	2

Legends: + = infected
- = negative
numeral = no. infected

TABLE VI

HAEMOFLAGELLATES AND INTESTINAL FLAGELLATES
OF LOUISIANA ANURA, LISTED AS TO HOSTS

PROTOZOAN	HOST-SPECIES													
	Hyla cinerea		Hyla crucifer		Hyla avivoca		Hyla femoralis		Hyla gratiosa		Hyla squirella		Hyla versicolor	
	Exam.	+	Exam.	+	Exam.	+	Exam.	+	Exam.	+	Exam.	+	Exam.	+
Trypanosoma karyozeukton	32	-	25	-	40	-	3	-	11	-	2	-	10	-
Trypanosoma rotatorium	32	2	25	1	40	1	3	-	11	-	2	-	10	-
Retortamonas dobelli	32	-	25	-	40	-	3	-	11	-	2	-	10	-
Chilomastix caulleryi	32	4	25	2	40	3	3	-	11	-	2	-	10	-
Karotomorpha swezyi	32	-	25	-	40	-	3	-	11	-	2	-	10	-
Monocercomonoides elegans n. sp.	32	-	25	-	40	-	3	-	11	-	2	-	10	-
Monocercomonoides melolonthae	32	-	25	4	40	-	3	-	11	-	2	-	10	1
Monocercomonas batrachorum	32	-	25	2	40	3	3	-	11	-	2	-	10	-
Tritrichomonas augusta	32	8	25	14	40	31	3	-	11	4	2	-	10	3
Tritrichomonas batrachorum	32	13	25	9	40	21	3	-	11	-	2	-	10	-
Trepomonas agilis	32	2	25	3	40	2	3	-	11	-	2	-	10	-
Urophagus intestinalis (?)	32	-	25	-	40	-	3	-	11	-	2	-	10	-
Hexamitus intestinalis	32	6	25	8	40	16	3	-	11	2	2	-	10	-
Octomastix batrachorum	32	-	25	12	40	17	3	-	11	-	2	-	10	-
Octomitus neglecta	32	-	25	8	40	15	3	-	11	-	2	-	10	-
Trimitus parvus	32	-	25	-	40	-	3	-	11	-	2	-	10	-

TABLE VII

HAEMOFLAGELLATES AND INTESTINAL FLAGELLATES
OF LOUISIANA ANURA, LISTED AS TO HOSTS

PROTOZOAN	HOST-SPECIES							
	Pseudacris nigrita		Pseudacris ornata		Microhyla carolinensis		Acris gryllus	
	Exam.	+	Exam.	+	Exam.	+	Exam.	+
Trypanosoma karyozeukton	40	-	1	-	17	-	50	5
Trypanosoma rotatorium	40	2	1	-	17	-	50	-
Retortamonas dobelli	40	3	1	-	17	-	50	5
Chilomastix caulleryi	40	6	1	-	17	-	50	11
Karotomorpha swezyi	40	-	1	-	17	-	50	-
Monocercomonoides elegans n. sp.	40	3	1	-	17	-	50	2
Monocercomonoides melolonthae	40	-	1	-	17	-	50	-
Monocercomonas batrachorum	40	6	1	-	17	-	50	4
Tritrichomonas augusta	40	16	1	-	17	11	50	34
Tritrichomonas batrachorum	40	11	1	-	17	6	50	16
Trepomonas agilis	40	3	1	-	17	-	50	4
Urophagus intestinalis (?)	40	-	1	-	17	3	50	-
Hexamitus intestinalis	40	17	1	-	17	4	50	11
Octomastix batrachorum	40	11	1	-	17	-	50	23
Octomitus neglecta	40	16	1	-	17	2	50	14
Trimitus parvus	40	-	1	-	17	-	50	-

TABLE VIII

HAEMOFLAGELLATES AND INTESTINAL FLAGELLATES
OF LOUISIANA ANURA, LISTED AS TO HOSTS

PROTOZOAN	HOST-SPECIES							
	Scaphiopus holbrookii		Bufo terrestris		Bufo valliceps		Bufo fowleri	
	Exam.	+	Exam.	+	Exam.	+	Exam.	+
Trypanosoma karyozeukton	50	-	20	-	38	-	20	-
Trypanosoma rotatorium	50	-	20	-	38	-	20	-
Retortamonas dobelli	50	-	20	2	38	3	20	1
Chilomastix caulleryi	50	-	20	4	38	-	20	-
Karotomorpha swezyi	50	-	20	3	38	-	20	2
Monocercomonoides elegans n. sp.	50	-	20	-	38	-	20	-
Monocercomonoides melolonthae	50	-	20	-	38	-	20	-
Monocercomonas batrachorum	50	-	20	-	38	-	20	2
Tritrichomonas augusta	50	12	20	8	38	15	20	7
Tritrichomonas batrachorum	50	17	20	11	38	19	20	9
Trepomonas agilis	50	-	20	-	38	-	20	-
Urophagus intestinalis (?)	50	-	20	3	38	-	20	-
Hexamitus intestinalis	50	-	20	6	38	11	20	7
Octomastix batrachorum	50	-	20	8	38	12	20	6
Octomitus neglecta	50	-	20	6	38	10	20	3
Trimitus parvus	50	-	20	-	38	2	20	1

SUMMARY

Information has been presented on the haemoflagellates and intestinal flagellates of Louisiana Anura. A total of 503 frogs and toads, representing 21 species, was collected and examined from 1953 to 1958.

The hosts were collected from 30 different localities throughout the state; however, the largest number was collected in the vicinity of Baton Rouge and adjacent areas.

Sixteen species of flagellates have been identified and studied. One of these has been described as a new species.

The haemoflagellates were stained with Giemsa. The intestinal flagellates were stained and impregnated with iron-haematoxylin and silver-protein.

The following haemoflagellates and intestinal flagellates are described:

Trypanosoma karyozeukton Dutton and Todd, 1903, was found in the host-species Acris gryllus crepitans. The morphology of this haemoflagellate as it appears with Giemsa is compared with earlier descriptions. It is considered a valid species.

Trypanosoma rotatorium (Mayer) 1843 is polymorphic, with widely variable individuals occurring in the same host. It differs from T. karyozeukton in being highly polymorphic, in the shape and position of the nucleus, and in the location of the blepharoplast. This species was found in 10 different species of Anura.

Retortamonas dobelli (Bishop) 1931 is described. The morphology of this flagellate compares favorably to the forms identified as R. dobelli of Bishop (1931) and Wenrich (1933). The species was found in eight host-species.

Chilomastix caulleryi (Alexeieff) 1909 is reported from nine species of Anura. Chilomastix caulleryi populations constitute two definite size races. No distinct morphological differences were noted between the size races; therefore, the two forms are considered to be one species. The large, robust race is compared with Chilomastix giganta Nie and found to have distinguishing features which set it apart from the latter species.

Karotomorpha swezyi (Grassé) 1926 is described. A comparison of the morphological characteristics of this species is made with iron-haematoxylin and silver-protein methods. The thick periplast, marked by striations which extend obliquely across the body, is clearly brought out

with silver-protein; however, this characteristic escapes observation in iron-haematoxylin specimens. The large, sausage-shaped structure which lies adjacent to the nucleus, and which Swezy (1916) specified as the parabasal body, is not impregnated by silver-protein and therefore is not comparable to the parabasal bodies of the trichomonad group.

Monocercomonoides elegans n. sp. is the only new species encountered during the study. It was found in two host-species. M. elegans most nearly resembles M. lacertae (Tanabe) 1933 but differs insofar as it possesses a spike-like structure at the posterior portion of the slender axostyle when it is stained with silver-protein.

Monocercomonoides melolonthae (Grassi) 1879 is described. The morphology of the flagellate as it appears in iron-haematoxylin is compared with earlier descriptions. In addition, specimens were impregnated with silver-protein and a comparison made with specimens stained by iron-haematoxylin.

Monocercomonas batrachorum (Dobell) 1909 is reported from eight anuran host-species. The morphology of the species stained with iron-haematoxylin is compared with former descriptions. Specimens impregnated with silver-protein reveal morphological characteristics that have not

up to this time been detected in the species. The pelta is a cone-like extension which impregnates lightly except for the anterior-most border, which impregnates heavily. The four flagella arise from the base of the parabasal body or just anterior to the structure. The parabasal body is a rod-like structure 2 or 3 microns in length and has its origin at the basal granule complex.

Tritrichomonas augusta (Alexeieff) 1911 was the most common intestinal flagellate found in the study, appearing in 18 species of Anura. The morphology of this intestinal flagellate as it appears in iron-haematoxylin and silver-protein is compared with earlier descriptions.

Tritrichomonas batrachorum (Perty) 1852 occurred in 14 species of anuran hosts. It differs from T. augusta in possessing three anterior flagella which are definitely unequal; the axostyle is more slender, contains no endo-axostylar granules, and is not surrounded by rings at the posterior section of this structure. The parabasal body is V-shaped, whereas in T. augusta it is rod- or sausage-shaped.

Trepomonas agilis Dujardin, 1841, is reported from eight anuran host-species. The morphology of the species as it appears in iron-haematoxylin is compared with earlier

descriptions. In addition, specimens impregnated with silver-protein are described. The most conspicuous organelles are the two nuclei--each show as a sickle-shaped structure at the antero-lateral portion of the organism. Together they appear to form a horse-shoe-shaped structure with the apex of the arch being situated at the extreme anterior end of the organism.

Urophagus intestinalis (?) (Alexeieff) 1910 is a rare flagellate in anuran hosts. The species was found in five host-species. Urophagus intestinalis (?) is characterized by having twin axostyles widely separated from each other and having a funnel-shaped flare-out on these structures, located at the anterior portion of the posterior section of the body proper. These funnel-shaped developments give the impression that they are cytostomal openings. The form is believed to be identical with the species described and depicted by Alexeieff (1910), although he described the form from Motella tricirrate and M. musteta.

Hexamitus intestinalis Dujardin, 1841, occurred in 15 species of Anura. Conditions and circumstances under which this species was found indicate that the species H. ovatus Swezy, 1915, and Spironucleus elegans Lavier, 1936, may be actually forms of H. intestinalis. A detailed

account of the morphology is given. Silver-protein-impregnated specimens show the bases of the anterior flagella composed of rod-like structures. These structures are separated into two groups which appear as inverted Y-shaped constructions.

Octomastix batrachorum (Swezy) 1915 was found in twelve species of Anura. Octomastix batrachorum possesses certain structures demonstrable by the silver-protein-impregnation method that have not up to this time been reported in the species. The bases of the flagella are composed of rod-like structures which impregnate with silver-protein. The two large extranuclear bodies appear as hollow spherical to ovoid doughnut-like rings situated in the posterior region of the organism. They lie free in the cytoplasm between the two slender axostyles. The basal granules at times are connected to each other by a fine filament. Corrections and additional descriptions of the species were also given. Some discussion was devoted to its taxonomic status.

Octomitus neglecta (Lavier) 1936 is reported from 15 different species of Anura. O. neglecta possesses certain morphological features revealed by silver-protein impregnation that have not been reported before in the species.

These structures are discussed.

Trimitus parvus Grassé, 1932, is rare and sparse in each of the host-species examined. The organism was found in five anuran species. Iron-haematoxylin and silver-protein individuals show the organism devoid of an axostyle and parabasal body. Observations have been made that confirm Alexeieff's characterization of the genus Trimitus.

A section was given to the discussion of host-parasite relationships. In addition, a checklist of the haemoflagellates and intestinal flagellates was presented and the number of species of frogs and toads infected by each parasite was stated.

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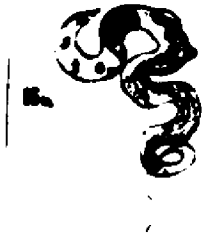

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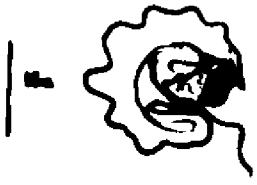

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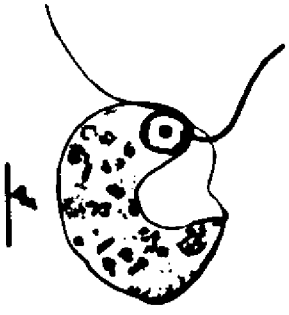

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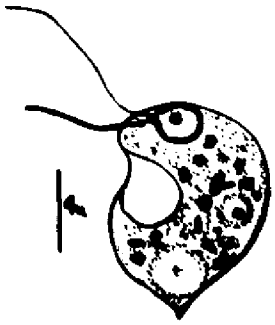

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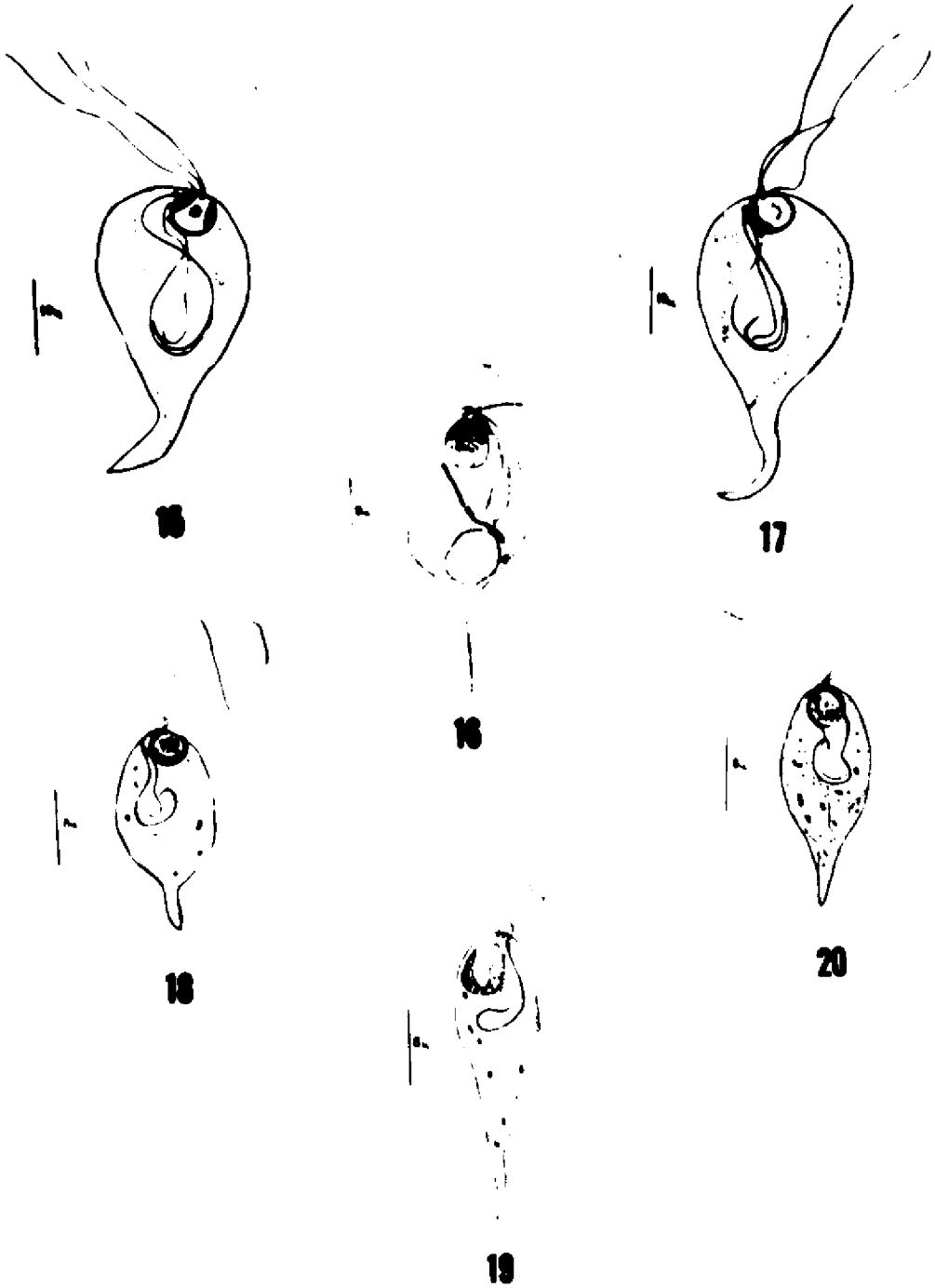

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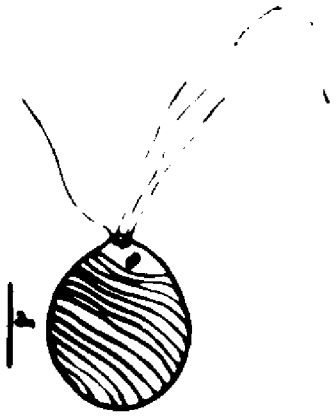

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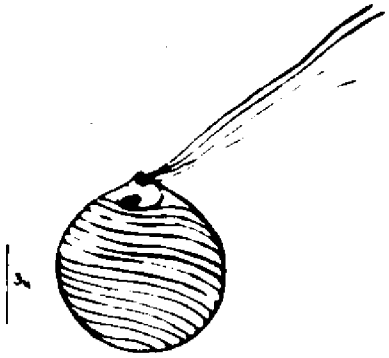

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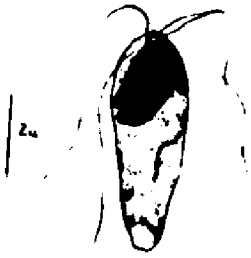

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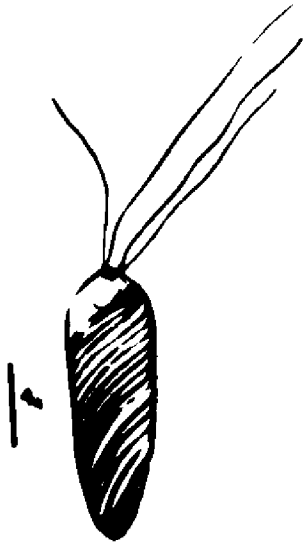

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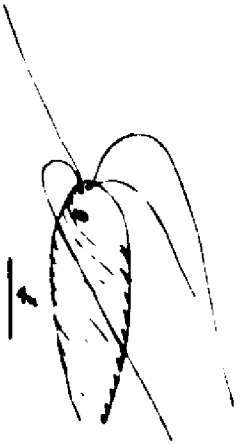

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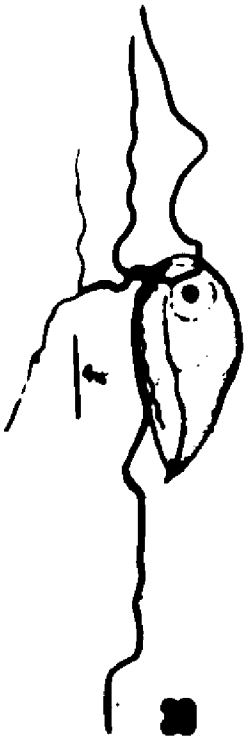

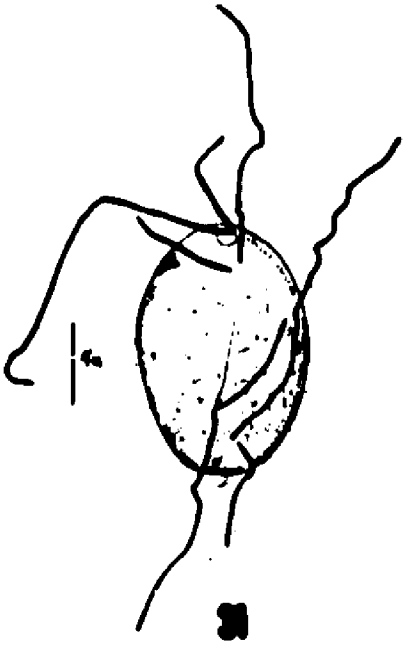

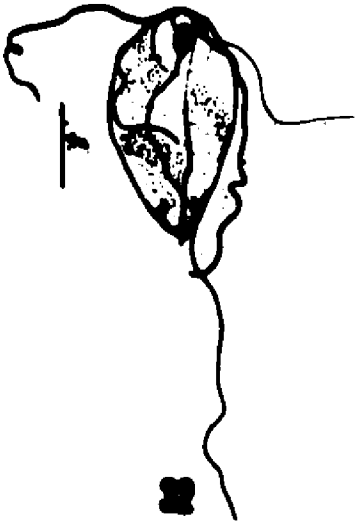

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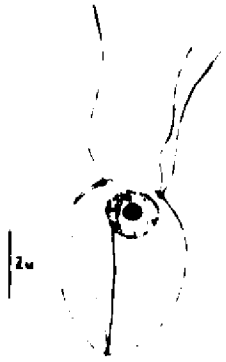

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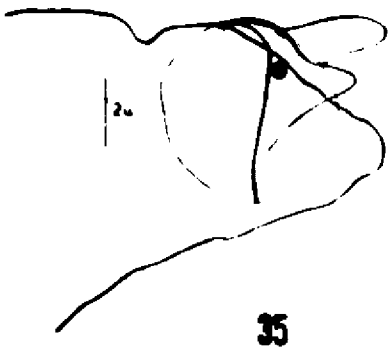

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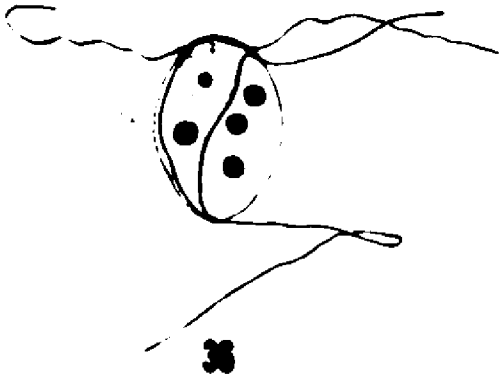

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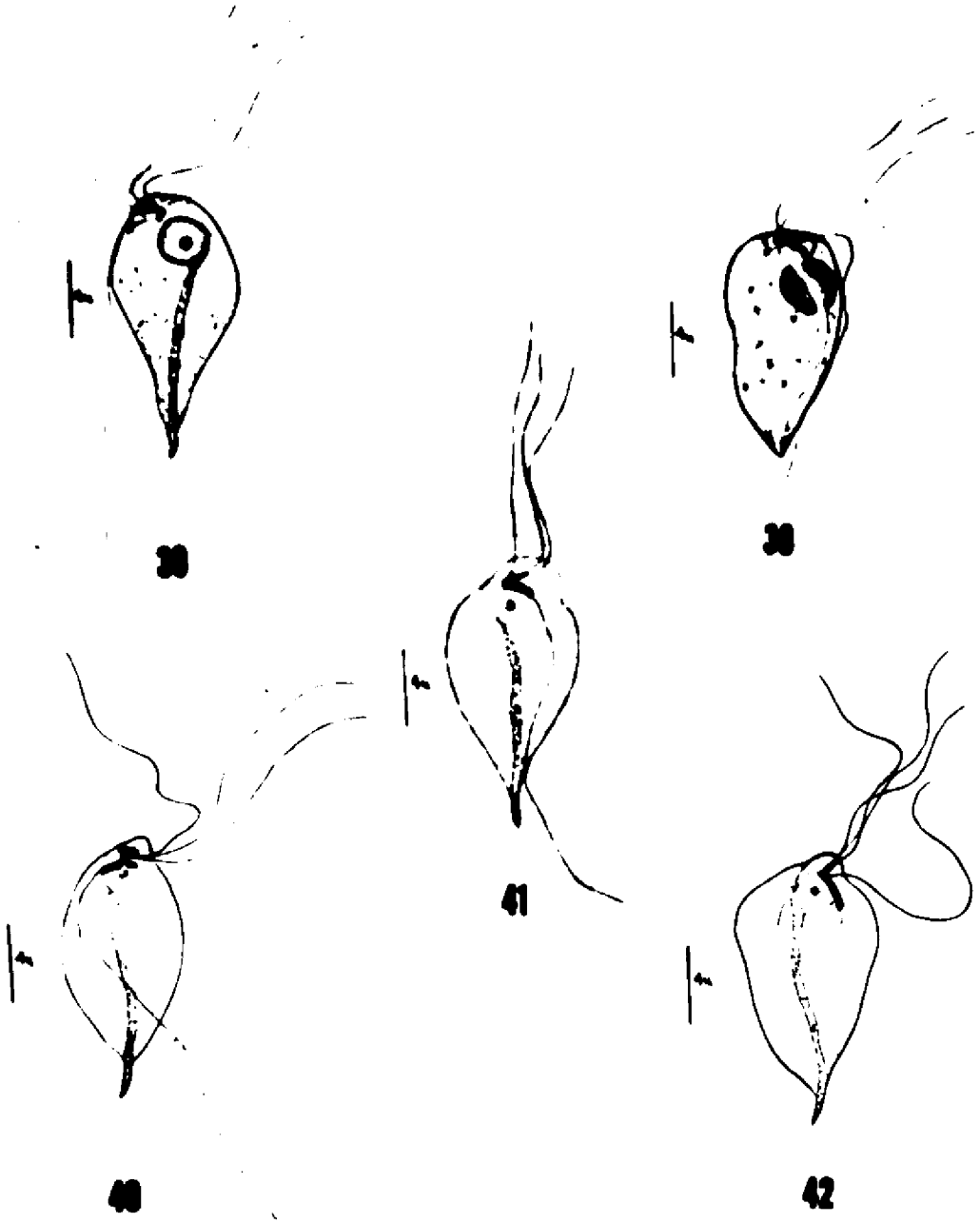

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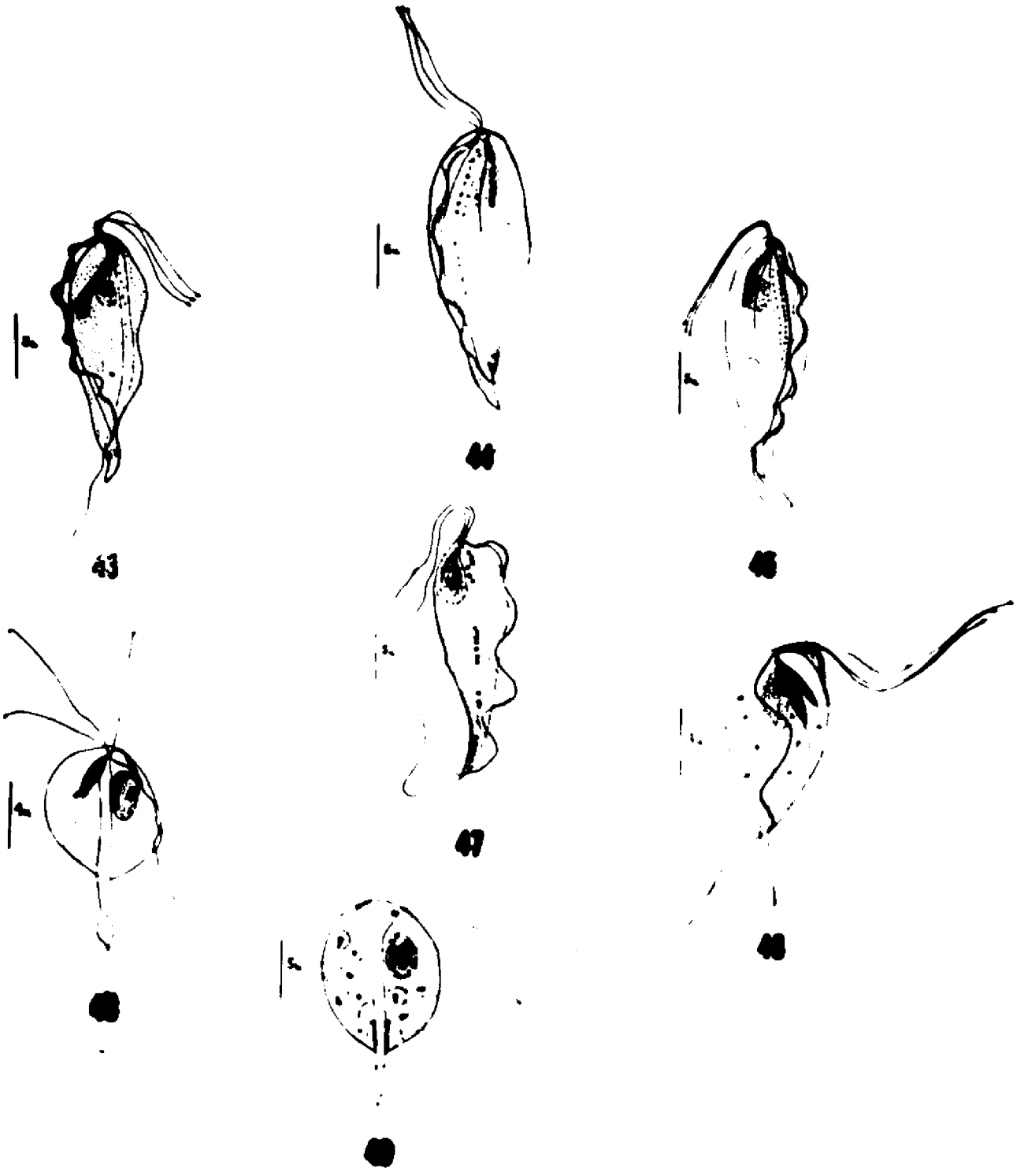

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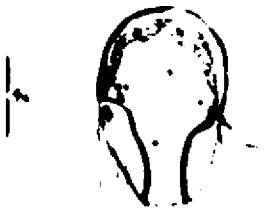

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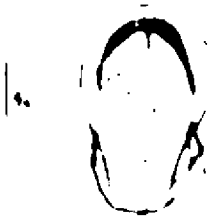

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Figure 59 Silver-Protein. *Urophagus intestinalis* (?) (Alexeieff) 1910

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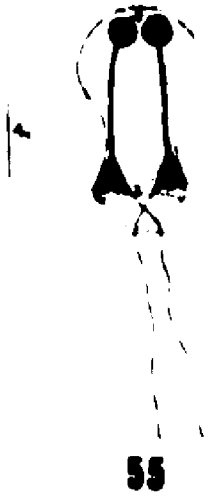

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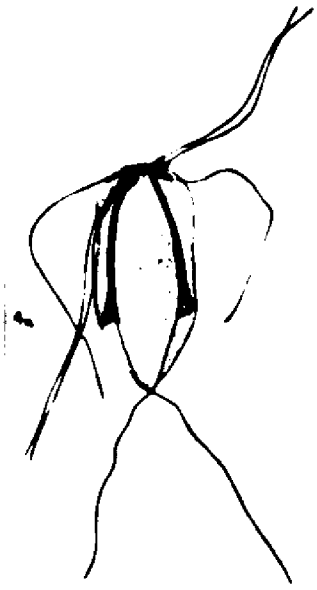

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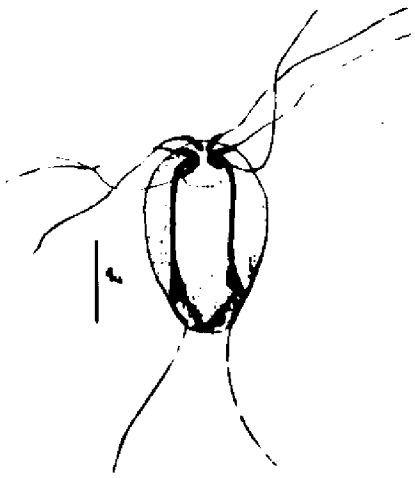

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Figure 67 Iron-Haematoxylin. *Octomastix batrachorum* (Swezy) 1915

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Figure 69 Silver-Protein. *Octomastix batrachorum* (Swezy) 1915

Figure 70 Silver-Protein. *Octomastix batrachorum* (Swezy) 1915

Figure 71 Silver-Protein. *Octomastix batrachorum* (Swezy) 1915

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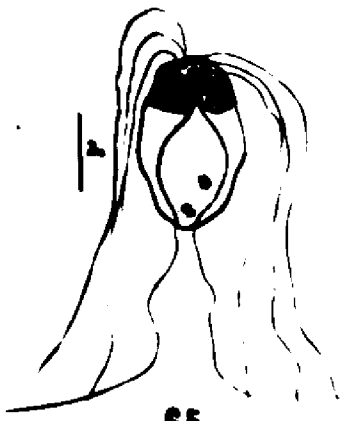

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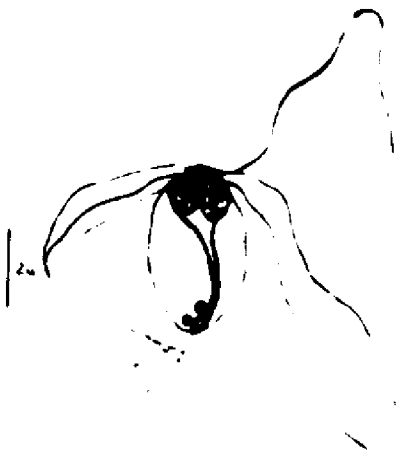

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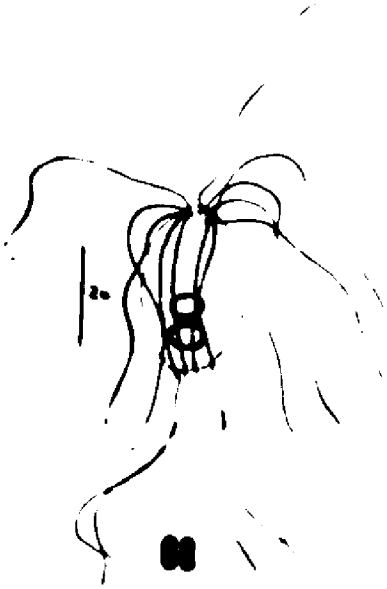

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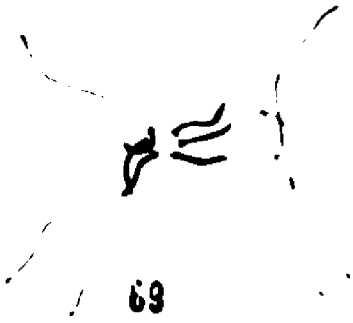

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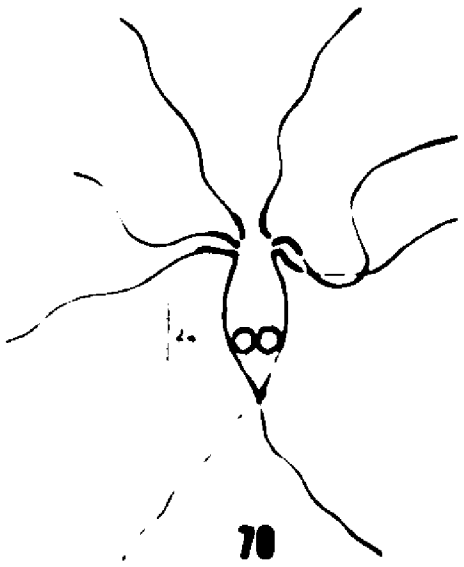

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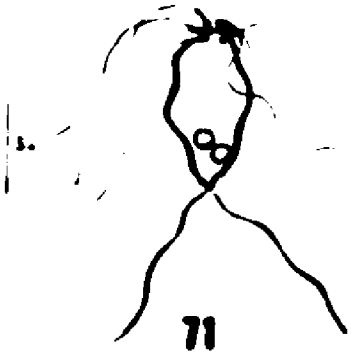

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Figure 73 Silver-Protein. *Octomitus neglecta* (Lavier) 1936

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Figure 75 Silver-Protein. *Octomitus neglecta* (Lavier) 1936

Figure 76 Silver-Protein. *Trimitus parvus* (Grassé) 1932

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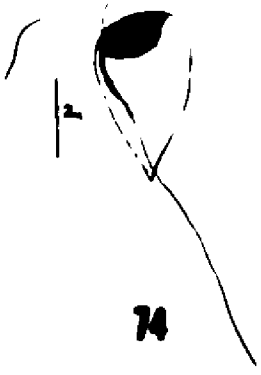

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VITA

Felix Hartwig Lauter was born at Somerville, New Jersey, February 12, 1919. He was graduated from Evanston Township High School, Evanston, Illinois, in June, 1937. He then attended Pennsylvania State College from September, 1937 to May, 1938, whereupon he accepted a sales position with France, Campbell and Darling, Inc., Kenilworth, New Jersey. He held this position until April, 1941, at which time he entered the United States Army and served overseas for two years. In December, 1945, he was released and returned to the position formerly held before entrance in the service. In 1948 he entered Southwestern College, Winfield, Kansas and received the degree of Bachelor of Arts in June, 1950. The following September he started graduate work at Louisiana State University. He received the degree of Master of Science in June, 1952. The following September he again entered Louisiana State University and started work on the degree of Doctor of Philosophy. From 1955 to 1957 he held a position as Assistant Professor of Biology at Birmingham-Southern College, Birmingham, Alabama. He then returned to Louisiana State University

in order to continue studies on the degree of Doctor of Philosophy. In 1958 he accepted a position as Assistant Professor of Biology at Illinois College, Jacksonville, Illinois. He currently holds this position.

He is now a candidate for the degree of Doctor of Philosophy in the Department of Zoology, Physiology and Entomology at Louisiana State University.

EXAMINATION AND THESIS REPORT

Candidate: Felix H. Lauter

Major Field: Zoology

Title of Thesis: Haemoflagellates and Intestinal Flagellates in Anura of Louisiana

Approved:

Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE:

Date of Examination:

July 20, 1959